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PROCEEDINGS

OF THE

ROYAL SOCIETY OF LONDON

Series B

CONTAINING PAPERS OF A BIOLOGICAL CHARACTER

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VOL. XC.

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HARRISON AND SONS, ST. MARTIN'S LANE,
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July, 1919.

LONDON:
HARRISON AND SONS, PRINTERS IN ORDINARY TO HIS MAJESTY
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CONTENTS.

SERIES B. VOL. XC.

No. B 623.—October 1, 1917.

PAGE

Observations and Experiments on the Susceptibility and Immunity of Rats towards Jensen's Rat Sarcoma. By J. C. Mottram, M.B. (Lond.), and Sidney Russ, D.Sc. (Lond.), Cancer Research Laboratories, Middlesex Hospital. Communicated by Prof. H. G. Plimmer, F.R.S. (Plates 1 and 2) 1

The Germicidal Action of Ultra-Violet Radiation, and its Correlation with Selective Absorption. By C. H. Browning, M.D., D.P.H., Director of the Bland-Sutton Institute of Pathology, Middlesex Hospital, and Sidney Russ, D.Sc., Physicist to the Middlesex Hospital. Communicated by Prof. A. W. Porter, F.R.S. (Plate 3) 33

The Isolation from Peat of Certain Nucleic Acid Derivatives. By W. B. Bottomley, M.A., Professor of Botany, University of London, King's College. Communicated by F. W. Oliver, F.R.S. 39

No. B 624.—December 1, 1917.

The Distribution in Wheat, Rice, and Maize Grains of the Substance, the Deficiency of which in a Diet causes Polyneuritis in Birds and Beri-beri in Man. By Harriette Chick and E. Margaret Hume. Communicated by Dr. C. J. Martin, F.R.S. 44

The Effect of Exposure to Temperatures at or above 100° C. upon the Substance (Vitamine) whose Deficiency in a Diet causes Polyneuritis in Birds and Beri-beri in Man. By Harriette Chick and E. Margaret Hume. Communicated by Dr. C. J. Martin, F.R.S. 60

Two Cases of Congenital Night-Blindness. By Sir W. de W. Abney, K.C.B., F.R.S. 69

No. B 625.—January 1, 1918.

The Enzymes Concerned in the Decomposition of Glucose and Mannitol by *Bacillus coli communis*. Part II.—Experiments of Short Duration with an Emulsion of the Organisms. By Egerton Charles Grey (Beit Memorial Research Fellow). Communicated by Dr. A. Harden, F.R.S. 75

PAGE

The Enzymes Concerned in the Decomposition of Glucose and Mannitol by *Bacillus coli communis.* Part III.—Various Phases in the Decomposition of Glucose by an Emulsion of the Organisms. By Egerton Charles Grey (Beit Memorial Research Fellow). Communicated by Dr. A. Harden, F.R.S. 92

The Germicidal Action of Ultra-violet Radiation and its Correlation with Selective Absorption.—Addendum. By C. H. Browning, M.D., and Sidney Russ.,D.Sc. Communicated by W. B. Hardy, Sec. R.S. 107

No. B 626.—April 2, 1918.

The Germicidal Action of Ultra-violet Radiation and its Correlation with Selective Absorption.—Addendum. By C. H. Browning, M.D., and Sidney Russ, D.Sc. Communicated by W. B. Hardy, Sec. R.S. 107

Croonian Lecture: Evolution and Symmetry in the Order of the Sea-pens. By Prof. Sydney J. Hickson, F.R.S. 108

Bactericidal Properties Conferred on the Blood by Intravenous Injections of Diamino-Acridine Sulphate. By C. H. Browning and R. Gulbransen. Communicated by Prof. R. Muir, F.R.S. 136

The Isolation and Serological Differentiation of *Bacillus tetani.* By W. J. Tulloch, M.D., Temp. Captain R.A.M.C. (Lecturer on Bacteriology at the University of St. Andrews). Communicated by Sir David Bruce, F.R.S., Chairman of the W.O. Committee for the Study of Tetanus 145

No. B 627.—June 1, 1918.

The Formation of Nitrites from Nitrates in Aqueous Solution by the Action of Sunlight, and the Assimilation of the Nitrites by Green Leaves in Sunlight. By Benjamin Moore, D.Sc., F.R.S. 158

Action of Light Rays on Organic Compounds, and the Photo-synthesis of Organic from Inorganic Compounds in Presence of Inorganic Colloids. By Benjamin Moore, D.Sc., F.R.S., and T. A. Webster 168

Growth of Trees, with a Note on Interference Bands formed by Rays at Small Angles. By A. Mallock, F.R.S. 186

On the Efficiency of Muscular Work. By M. Greenwood, Captain R.A.M.C. (T.F.), Lister Institute of Preventive Medicine. Communicated by Prof. Leonard Hill, F.R.S. 199

The Galvanometric Measurement of "Emotive" Physiological Changes. By A. D. Waller, M.D., F.R.S. 214

No. B 628.—July 1, 1918.

On the Quantitative Differences in the Water-Conductivity of the Wood in Trees and Shrubs. Part I.—The Evergreens. By J. Bretland Farmer, F.R.S., Professor of Botany in the Imperial College of Science and Technology 218

On the Quantitative Differences in the Water-Conductivity of the Wood in Trees and Shrubs. Part II.—The Deciduous Plants. By J. Bretland Farmer, F.R.S., Professor of Botany in the Imperial College of Science and Technology 232

PAGE
The Production of Anthocyanins and Anthocyanidins.—Part III. By Arthur Ernest Everest, D.Sc., Ph.D. Communicated by Dr. F. W. Keeble, F.R.S....... 251

Note on the Nature of Growths in Colloidal Silica Solutions. By H. Onslow, Trinity College, Cambridge. Communicated by Prof. F. G. Hopkins, F.R.S. 266

No. B 629.—October 17, 1918.

Brevity, Frequency of Rhythm, and Amount of Reflex Nervous Discharge, as indicated by Reflex Contraction. By N. B. Dreyer and C. S. Sherrington, F.R.S. 270

Croonian Lecture.—The Physiological Basis of Thirst. By Major Walter B. Cannon, M.R.C., U.S. Army; George Higginson Professor of Physiology, Harvard University 283

The Mechanism and Control of Fibrillation in the Mammalian Heart. By J. A. MacWilliam, F.R.S. 302

No. B 630.—December 20, 1918.

The Artificial Production of Echinoderm Larvæ with Two Water-Vascular Systems, and also of Larvæ Devoid of a Water-Vascular System. By E. W. MacBride, F.R.S. (Plates 4–10) 323

Changes of Electrical Conductivity under Geotropic Stimulation. By James Small, M.Sc. (Lond.), F.L.S. Communicated by Dr. A. D. Waller, F.R.S. 349

No. B 631.—February 17, 1919.

Researches on Growth and Movement in Plants by Means of the High Magnification Crescograph. By Sir Jagadis Chunder Bose, assisted by Guruprasanna Das, L.M.S. Communicated by Prof. S. H. Vines, F.R.S. 364

Investigations dealing with the State of Aggregation. Part IV.—The Flocculation of Colloids by Salts containing Univalent Organic Ions. By S. B. Schryver, D.Sc., and Nita E. Speer. Communicated by V. H. Blackman, F.R.S. 400

No. B 632.—April 1, 1919.

Observations on Changes in the Blood Pressure and Blood Volume following Operations in Man. (Preliminary Communication.) By Captain H. C. Bazett, M.C., R.A.M.C. Communicated by Prof. W. M. Bayliss, F.R.S. 415

On the Cooling and Evaporative Powers of the Atmosphere, as Determined by the Kata-thermometer. By Leonard Hill, F.R.S., and D. Hargood-Ash. 438

The Influence of External Concentration on the Position of the Equilibrium attained in the Intake of Salts by Plant Cells. By Walter Stiles, M.A., Lecturer in Botany in the University of Leeds, and Franklin Kidd, M.A., D.Sc., Fellow of St. John's College, Cambridge. Communicated by Prof. Bayliss, F.R.S. 448

PAGE

No. B 633.—May 15, 1919.

On the Four Visible Ingredients in Banded Bituminous Coal: Studies in the Composition of Coal, No. 1. By Marie C. Stopes, D.Sc., Ph.D., Fellow and Lecturer in Palæobotany, University College, London. Communicated by Sir George Beilby, F.R.S. (Plates 11 and 12) 470

The Comparative Rate of Absorption of Various Salts by Plant Tissue. By Walter Stiles, M.A., Lecturer in Botany in the University of Leeds, and Franklin Kidd, M.A., D.Sc., Fellow of St. John's College, Cambridge. Communicated by Prof. W. M. Bayliss, F.R.S. 487

Changes in the Teeth of the Guinea-pig, produced by a Scorbutic Diet. By S. S. Zilva, Ph.D., M.Sc., and F. M. Wells, Major, C.A.D.C. Communicated by Dr. A. Harden, F.R.S. (Plate 13) 505

On a New Factor in the Mechanism of Bacterial Infection. By W. E. Bullock and W. Cramer. Communicated by Prof. W. Bulloch, F.R.S. 513

No. B 634.—July 1, 1919.

The Distribution of the Serological Types of *B. tetani* in Wounds of Men who received Prophylactic Inoculation, and a Study of the Mechanism of Infection in, and Immunity from, Tetanus. By Major W. J. Tulloch, M.D., R.A.M.C., Lecturer in Bacteriology, University of St. Andrews, Member of the War Office Committee for the Study of Tetanus. Communicated by Major-General Sir David Bruce, F.R.S. 529

The Existence of Daily Growth-rings in the Cell Wall of Cotton Hairs. By W. Lawrence Balls, M.A., Sc.D. (Cantab.), late Fellow of St. John's College, Cambridge. Communicated by F. F. Blackman, F.R.S. (Plates 14, 15 and 16) 542

CROONIAN LECTURE.—The Biological Significance of Anaphylaxis. By Dr. H. H. Dale, F.R.S. (Abstract.) 556

OBITUARY NOTICES OF FELLOWS DECEASED.

Sir William Henry Power i
Clement Reid (with portrait) viii
Daniel Oliver (with portrait) xi
Captain Geoffrey Watkins Smith xvi
Hermann Graf zu Solms-Laubach (with portrait) xix
Robert Bell........ xxvi
Edward Hull (with portrait) xxviii
Keith Lucas (with portrait)........ xxxi
Henry George Plimmer (with portrait) xliii
Alfred Merle Norman (with portrait) xlvi

Index li

PROCEEDINGS
OF
THE ROYAL SOCIETY.

Series B. Vol. 90. **No. B 623.**

BIOLOGICAL SCIENCES.

CONTENTS.

Page

Observations and Experiments on the Susceptibility and Immunity of Rats towards Jensen's Rat Sarcoma. By J. C. MOTTRAM, M.B. (Lond.), and SIDNEY RUSS, D.Sc. (Lond.), Cancer Research Laboratories, Middlesex Hospital. (Plates 1 and 2) 1

The Germicidal Action of Ultra-Violet Radiation, and its Correlation with Selective Absorption. By C. H. BROWNING, M.D., D.P.H., Director of the Bland-Sutton Institute of Pathology, Middlesex Hospital, and SIDNEY RUSS, D.Sc., Physicist to the Middlesex Hospital. (Plate 3) . 33

The Isolation from Peat of Certain Nucleic Acid Derivatives. By W. B. BOTTOMLEY, M.A., Professor of Botany, University of London, King's College 39

Obituary Notices of Fellows deceased:—Sir William Henry Power; Clement Reid (with portrait) . . . . i—x

PRINTED FOR THE ROYAL SOCIETY AND SOLD BY
HARRISON & SONS, 45, ST. MARTIN'S LANE, LONDON, W.C.

Price Three Shillings and Sixpence.

No. B 623.

October 1, 1917.

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PROCEEDINGS OF

THE ROYAL SOCIETY.

Section B.—Biological Sciences.

Observations and Experiments on the Susceptibility and Immunity of Rats towards Jensen's Rat Sarcoma.

By J. C. Mottram, M.B. (Lond.), and Sidney Russ, D.Sc. (Lond.), Cancer Research Laboratories, Middlesex Hospital.

(Communicated by Prof. H. G. Plimmer, F.R.S. Received November 9, 1916.)

[Plates 1 and 2.]

CONTENTS.

Part I.

Page.
Conditions of Susceptibility and Immunity 2
  1. The Condition of Susceptibility 2
  2. The Condition of Immunity 4
    (*a*) General 4
    (*b*) Production of Immunity 4
    (*c*) Condition of Tissues 7
  3. The Effect of Experimental Alteration of Susceptible and Immune Animals upon Subsequent Inoculation 10
    (*a*) Splenectomy 10
    (*b*) Large Inoculation 10
    (*c*) Exposure to X-rays 11
  4. The Effect of Experimental Alteration of the Sarcoma Cell before Inoculation into Susceptible and Immune Animals 13
    (*a*) Mixture of Sarcoma Cells with Spleen and Liver, etc. 13

Part II.

Microscopic Observations upon Grafts and Tumours 16
  1. The Study of the Graft in Susceptible Animals 17
  2. The Study of the Graft in Immune Animals 20
  3. The Study of the Graft in Animals which have been Experimentally Altered before Inoculation 22
  4. The Study of the Graft in Animals Inoculated with Altered Sarcoma Cells 23
  5. The Study of Regressing and Oscillating Tumours 24
  6. Comparison of Results 25

Part III.

General Discussion and Conclusions 28

Part I.—Conditions of Susceptibility and Immunity.

1. *The Condition of Susceptibility.*

The Jensen's rat sarcoma used in this investigation was kindly provided by the Imperial Cancer Research Fund, and has been in continual propagation in this laboratory during the past five years.

The inoculation of normal rats with this sarcoma results in the large majority of cases in a growing tumour. The behaviour of the tumour has been investigated in detail.

Four types of growth are clearly distinguishable; they are :—

(1) Cases in which the rate of growth of the tumour is uniform; these are referred to as "Progressive" tumours.

(2) Cases in which the rate of growth gradually diminishes as the tumour increases in size; these are referred to as "Retarded" tumours.

(3) Cases in which, after attaining a certain size, the tumour oscillates between narrow limits for long periods of time (often for months); these are referred to as "Oscillating" tumours.

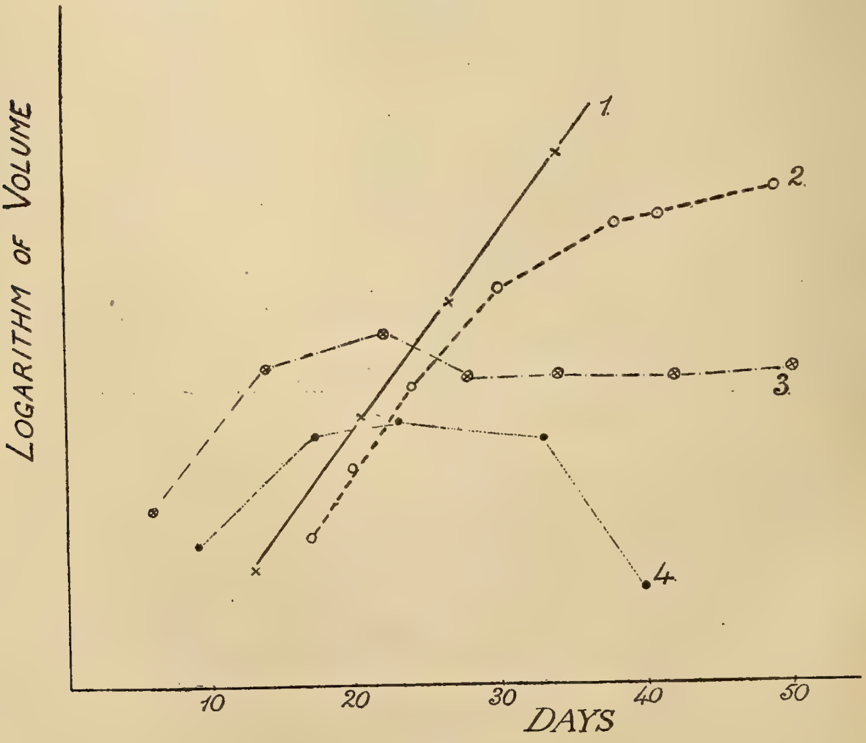

Fig. 1.

(4) Cases in which the tumour spontaneously disappears; these are referred to as "Disappearing" tumours.

The test as to whether a tumour belongs to type 1 or 2 is to make frequent measurements of the superficial area of the tumour. If these areas are raised to the power 3/2 and their logarithms when plotted are found to lie on a straight line, then the rate of growth of the tumour has been uniform; a typical case may be seen in (1), fig. 1. No. 2 illustrates the gradual diminution in the rate of growth which serves to distinguish type 2 from type 1. Nos. 3 and 4 illustrate the oscillating and disappearing types respectively.

The probability of success on re-inoculating animals which are bearing or have borne tumours of any of the types specified may be gauged from the data in Table I.

Table I.

Character of tumour.	No. of rats.	Result of re-inoculation.	Subsequent behaviour of tumour.
Progressive ...	18	Graft took	Progressed.
	2	,, ,,	Retarded.
	2	,, ,,	Recurred after operation.
	1	Graft took but regressed	Recurred after operation, then regressed.
	1	Graft did not take	Retarded.
	1	,, ,, ,,	Recurred after operation, then regressed.
	2	,, ,, ,,	Did not recur after operation.
Retarded	1	Graft took	Progressed.
	1	Graft took but disappeared	Retarded.
	1	Graft did not take	Progressed, then retarded.
	3	,, ,, ,,	Oscillatory.
	2	,, ,, ,,	Retarded.
	3	,, ,, ,,	Disappeared.
	1	,, ,, ,,	Recurred but regressed.
	1	,, ,, ,,	Did not recur after operation.
Oscillating ...	1	Graft did not take	Oscillated for six weeks, then progressed.
	1	,, ,, ,,	Oscillated.
	4	,, ,, ,,	Gradually disappeared.
	1	Graft took	Oscillated.
	1	,, ,,	No recurrence after operation.
Disappearing	1	Graft took	Subsequently grew.
	8	Graft did not take	Disappeared.
	5	,, ,, ,,	Did not recur after operation.
	1	Graft took	Subsequently disappeared.
	21	Graft did not take	,, ,,

Judging from the behaviour of this strain of sarcoma in about 2000 inoculations during the last four years, it is estimated that about 70 per cent. of inoculations yield growing tumours of types 1 and 2, about 5 per cent. of an oscillating nature, and 25 per cent. which spontaneously disappear. These latter often attain to considerable size; tumours measuring as much as 6 sq. cm. have subsequently been observed to disappear.

2. *The Condition of Immunity.*

(a) *General.*—Rats are rarely found to be immune to a first inoculation of the sarcoma cells. The animals in which the resulting tumours spontaneously regress are almost invariably resistant to a second inoculation; when tested two or three months later, they are generally in a similar state. Our studies upon the immune condition have been largely made upon rats which at one time have borne tumours, and their resistant nature proved by the failure of a second inoculation.

The wide variations observed in the behaviour of the inoculated sarcoma cells are not due to differences in the inoculated material. A progressively growing tumour may be removed from an animal, made into a uniform emulsion and inoculated into a batch of, say, 20 normal rats of about 100 grm. weight each. It may be fairly confidently stated that the subsequent tumours will furnish examples of all four types of growth which have been described; this being so, it is rational to attribute the varying fate of the sarcoma cells to the defensive mechanism which the rats are able to bring to bear against these cells. This defensive mechanism is one which may eventually overpower the growing sarcoma cells, causing their complete disappearance (*vide* type 4); such animals are immune to subsequent inoculation, and the sarcoma cells of tumours in the course of disappearing are rarely transplantable with success (*vide* fig. 2). The oscillating tumours indicate a condition in which the growth of the cells is just balanced by the defensive mechanism; animals bearing such tumours are generally found to be immune to a subsequent inoculation. The transplantation of oscillating tumours gives a moderate number of growing tumours (*vide* fig. 3).

With still smaller degrees of defensive power on the part of the animal there appear retarded tumours, and, lastly, progressive tumours. In these last named, the defensive mechanism is not always entirely absent, for re-inoculation of the animals does not invariably result in growing tumours. Recourse is naturally had to tumours of type 1 or 2 for the continued propagation of the tumour.

(b) *Production of Immunity.*—If sarcoma cells are exposed to the β-rays from radium under suitable conditions of exposure (1), it is found that when the irradiated cells are inoculated into normal rats they do not develop into tumours. If the degree of irradiation is not too prolonged, the animals are frequently found to be immune to a fresh inoculation of normal sarcoma cells.

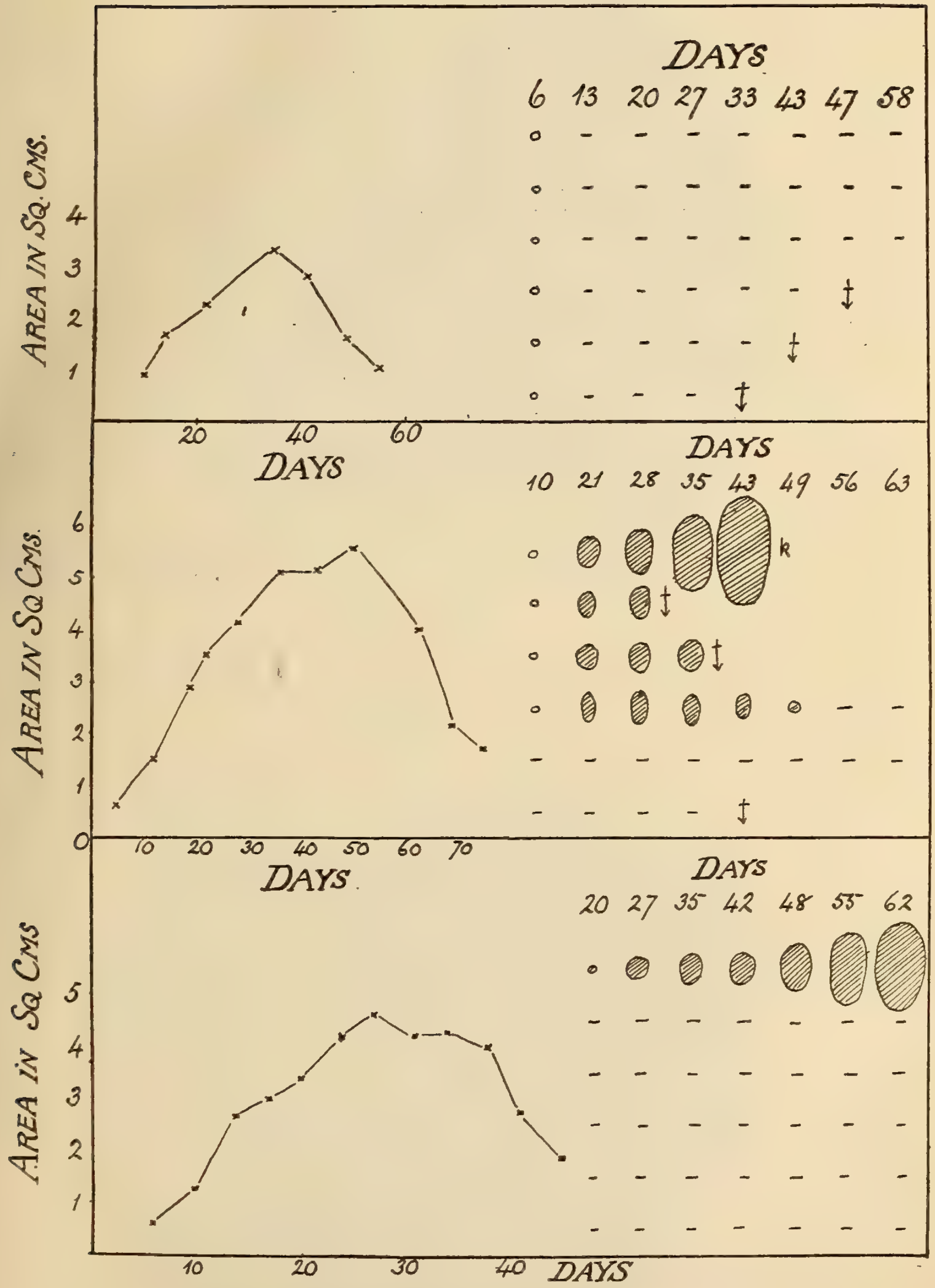

FIG. 2.—The plotted line shows the tumour growth up to the day of inoculation. The result of the inoculation is seen in the charts to the right.

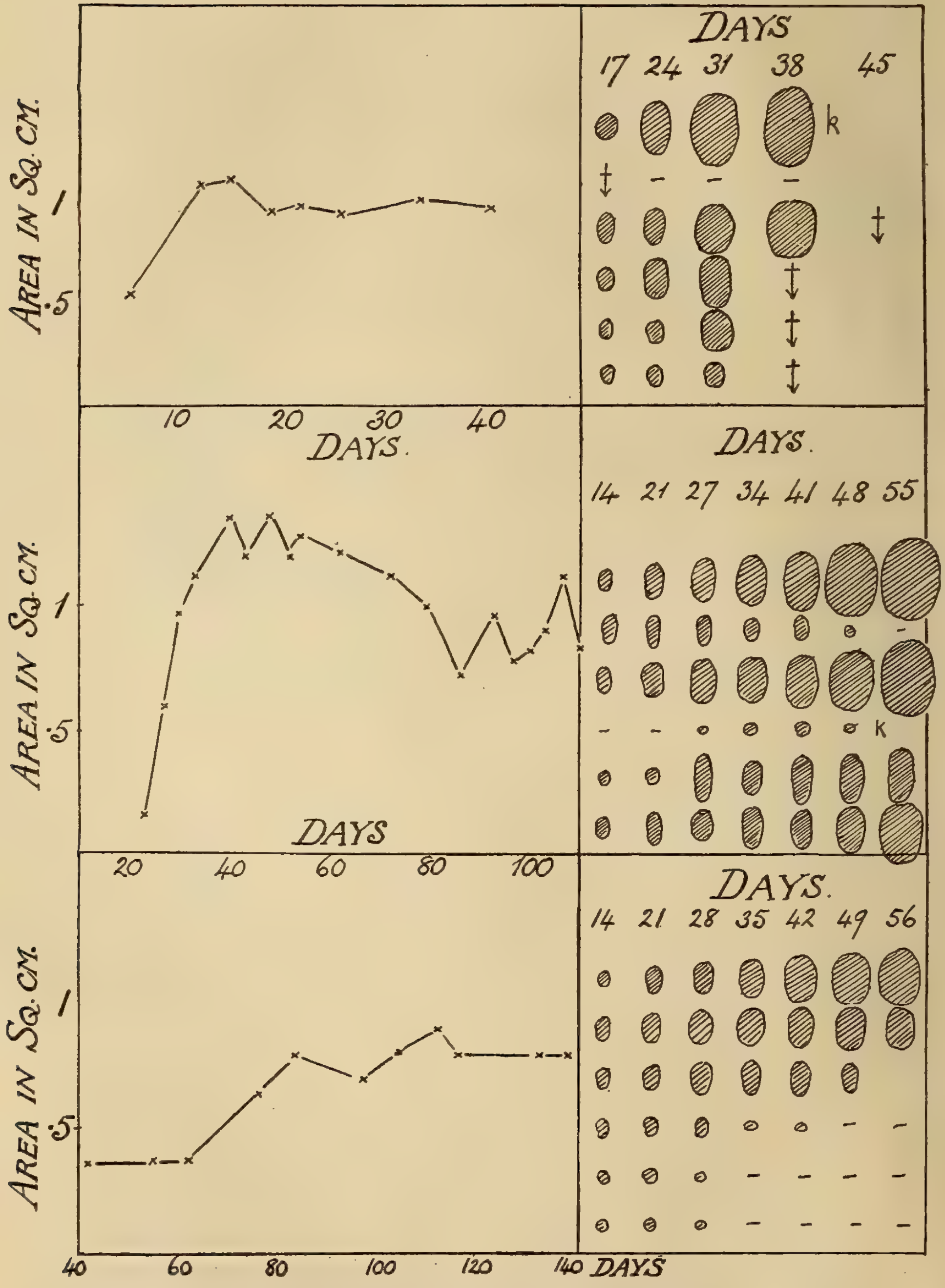

Fig. 3.—The plotted line shows the tumour growth up to the day of inoculation. The result of the inoculation is seen in the charts to the right.

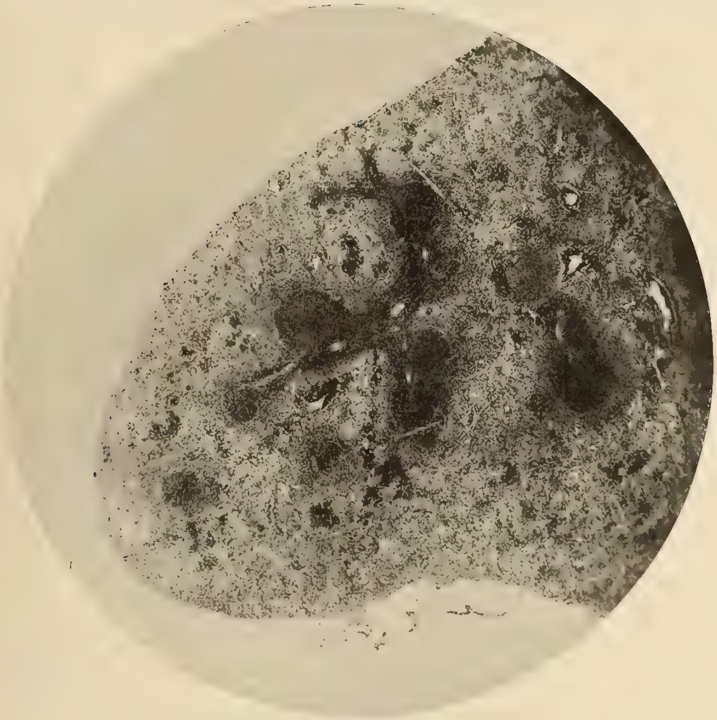

Fig. 4 (Normal Spleen).

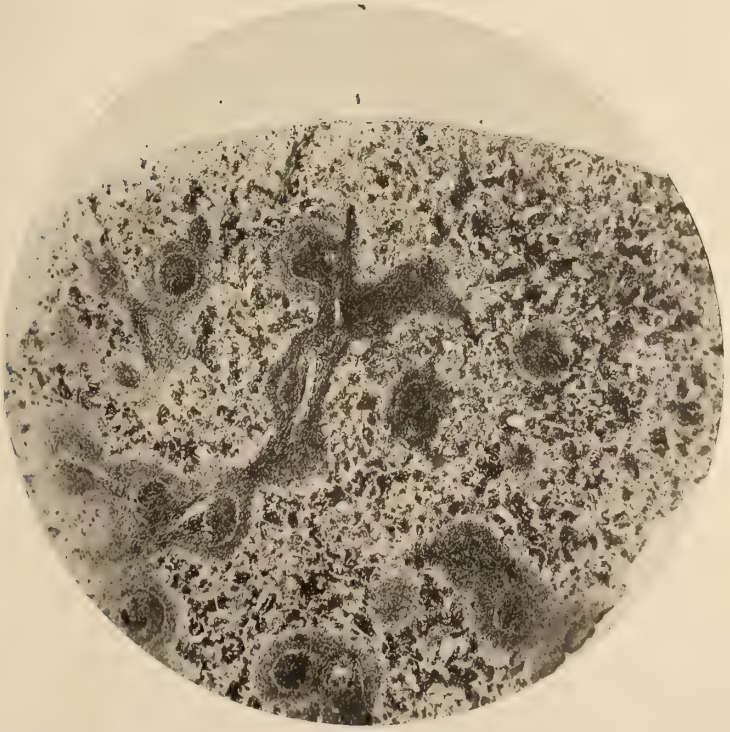

Fig. 5 (Immune Spleen).

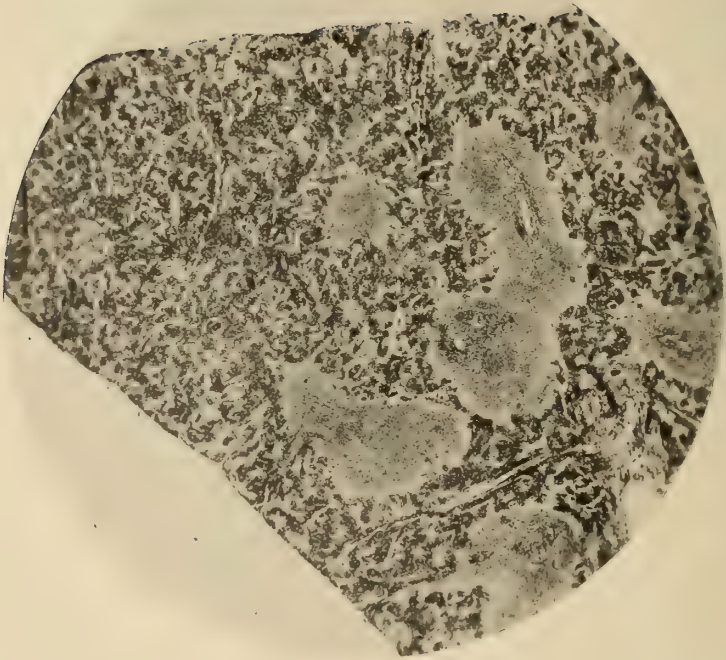

Fig. 6 ("Massive" Spleen).

Rats may be made immune in a similar manner if the sarcoma cells are previously exposed to radium emanation in a concentration of about 0·5 millicurie per c.c., the exposure lasting about one hour (*loc. cit.*). The amount of irradiated material inoculated has usually been 0·5 c.c. in the right and left axillæ; after two or three weeks the immunity of the animals is tested by inoculation.

(c) *Condition of Tissues.*—A microscopic examination of the spleen shows, in a very large number of cases, a striking difference between the normal and the immune animal. This contrast is provided by a very large increase in the total number of lymphocytes and plasma cells in the spleen of immune animals compared with that usually obtaining in the normals. (This increase was found to be accentuated in the immune rats after they had received a large inoculation, *i.e.*, 0·3 or 0·4 c.c.) Other observers have shown a similar increase in these cells throughout the connective tissues (2).

Figs. 4, 5, 6 (Plates 1 and 2) illustrate the contrast referred to in the three types of spleen.

This contrast in the appearance of the spleen seemed to warrant a more extended investigation. The attempt was made to see whether this massing of the lymphocytes and plasma cells was directly associated with the development of the immune condition.

In the first place, an examination of the spleen was made in a large number of animals which were in different conditions as regards their toleration to the growth of sarcoma cells. They were classified as Normal (N), *i.e.*, rats practically certain to bear tumours if inoculated; Immune (I), *i.e.*, rats proved to be resistant to inoculation; "Massive" (M), *i.e.*, immune rats which had been inoculated with 6 × 0·05 c.c. sarcoma emulsion with negative results; Progressive (P), *i.e.*, animals which were supporting the growth of a progressively growing tumour; and Disappearing (D), *i.e.*, rats in which tumours were spontaneously disappearing.

By giving numerical values, from 0 to 12, to the lymphocyte and plasma-cell content of the spleen, it was possible to classify these various types, and to show that an increased content was not necessarily associated with the immune condition, nor did an immune animal invariably exhibit a high content of these particular cells in its spleen, although this was the usual condition.

To eliminate all personal bias in the matter, the whole collection of slides (numbering about 300) of the sections of the various spleens was examined without any knowledge on the part of the observer of the particular class to which any slide belonged. Numerical values of the cell content were written on the slides, which were then tabulated. The results are shown graphically in fig. 7.

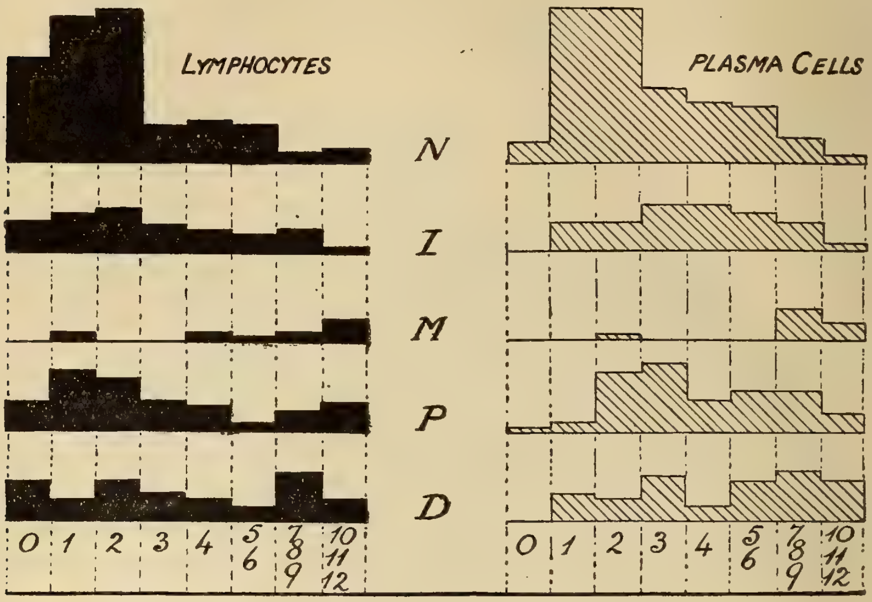

Fig. 7.

The finding of an occasional normal animal, or one bearing a progressively growing tumour with a very high lymphocyte and plasma-cell content, does not, however, rule out the possibility that in such cases these cells may be playing some part in what may still be called immunity. Reference to Table I shows that a small number of animals, exhibiting all the outward signs of susceptibility, simultaneously exhibit an immunity to the graft, for they resist re-inoculation.

The question remained, therefore, as to whether these few animals corresponded with the small proportion of their class, giving a high lymphocyte and plasma-cell count. This was put to the test direct in the following way: A batch of normal rats was taken, and a small portion of their spleens removed; they were then given an inoculation into the right axilla.

The animals grew tumours, of which frequent records were kept; when at least four measurements upon each rat had been made, selection was made of those which had progressive tumours (Type 1); a further portion of their spleens was then removed, and their lymphocyte and plasma-cell content again determined.

It was found that the majority, 19 out of 22, of the rats growing progressive tumours were positive to a fresh inoculation, and that in some of these cases a considerable increase in their leucocyte and plasma-cell content occurred; in other animals which were resistant to a re-inoculation, although bearing progressive tumours, there was no appreciable increase in the lymphocyte and plasma-cell content directly associated with the appearance of the immune condition.

The outstanding features of the investigation are:—

(1) The lymphocyte and plasma-cell content of normal rats varies within wide limits, but the number having a low content is very much larger than the number having a high content [*vide* diagram (N)].

(2) The immune animal is just about as likely to have a low as a high content (I); "Massive" immunes (*vide* fig. 7) are almost always packed with cells of the kind considered (M).

(3) There is about an equal chance in animals which bear either a progressive or a disappearing tumour, having a high or low cell content (P), (D).

Any observed increase in the lymphocyte and plasma-cell content is not due to intolerance of the spleen to repeated interference with it—this was shown by separate tests upon normal rats. Neither can the variations be induced by inoculating dead cells into the animals; this was tested by inoculating boiled sarcoma cells with two subsequent examinations of the spleen content—no appreciable variation of the lymphocyte and plasma-cell content occurred.

Tumours in Living Rat Tissues before Inoculation.—Microscopical examination [*vide* p. 20 (*d*)] shows that the sarcoma cells can no longer be found after five or six days in the immune rat. If, however, the graft be removed at shorter intervals and re-inoculated into normal rats, the sarcoma cells are still viable in a fair percentage of cases, after having been in an immune animal for as long a period as three days (*vide* Table II).

Protocol.—0·05 c.c. of minced tumour was placed in the axillæ and spleens

Table II.

No. of days the tumour was left in the normal and immune rats.	The growth of the tumour after having been in the axillæ of immune rats.	The growth of the tumour after having been in the axillæ of normal rats.	The growth of the tumour after having been in the spleen of immune rats.	The growth of the tumour after having been in the spleen of normal rats.
1	8/12	8/12	8/12	6/12
2	—	—	5/9	8/9
3	6/20	17/21	10/18	18/21
6	0/11	90–100 per cent.	0/11	90–100 per cent.

of normal and immune rats. After varying lengths of time this was removed; small pieces were then taken from the margin (avoiding as far as possible the necrotic centre), and placed in the axillæ of normal rats. The denominators of the fractions give the number of rats inoculated, the numerators the number of rats in which the inoculation resulted in measurable tumours.

Result.—The tumour was not killed nor its subsequent growth affected by exposure for three days to the living subcutaneous or splenic tissues of immune rats. Exposure for six days to the tissues of immune rats prevented the tumour subsequently growing.

3. *The Effect of Experimental Alteration of Susceptible and Immune Animals upon Subsequent Inoculation.*

(a) *The Influence of Splenectomy upon Tumour Growths.*—The study of the modes of growth of the sarcoma, and the observance of the immunity thereto, led to a search being made for the factors which control this latter condition.

Attention was directed to the spleen, and a preliminary investigation was made by extirpating this organ in normal and immune rats and seeing whether any change was induced to the subsequent fate of sarcoma when inoculated into such animals.

Normal and immune rats were splenectomised and then inoculated with 0·05 c.c. of sarcoma at periods ranging from 0 to 28 days after the operation. The data in Table III show that the splenectomy of normal rats does not affect the subsequent growth of the tumour in them; it shows also that in immune rats the act of splenectomy does not abolish the immunity to a subsequent graft. Brancati (3) and Apolant (4) have shown that with rat and mouse carcinoma, splenectomy favours growth in normal animals.

(b) *The Effect of a Large Inoculation.*—Whatever view may be held as to

Table III.

	No. of rats.	Result of inoculation.
Splenectomised normal rats …………	26	17 gave progressive tumours. 4 gave disappearing tumours. 5 gave oscillatory tumours.
Splenectomised immune rats …………	24	All negative.
Control rats not splenectomised ………	35	22 gave progressive tumours. 10 gave disappearing tumours. 3 gave oscillatory tumours.

the nature of the processes operative in immune animals it seemed advisable to see whether by a large inoculation the immune condition could be overcome. For this purpose 50 immune rats were given six inoculations of 0·05 c.c. of sarcoma cells in the subcutaneous tissues of the flanks and abdomen. In the majority of these small nodules could be felt for 12 to 14 days; in only two cases did they attain sufficient size to be measured.

Twenty immune rats were splenectomised and were then given six or eight inoculations of 0·05 c.c. of tumour. In several cases persistent nodules resulted: in one case a small tumour grew, but subsequently regressed; in another case six large tumours resulted in a single animal.

(c) *Exposure to X-Rays.*—When an animal such as a rat is exposed *in toto* to X-rays, a moderate degree of irradiation is sufficient to produce changes in its blood which can easily be recognised. The white cells are more affected by these rays than are the red cells, and of the white cells the lymphocytes are especially vulnerable; by prolonged irradiation the circulating blood may be temporarily rid of practically all its lymphocytes.

The work which has been described shows that the lymphocytes play some part in the processes by which immunity to the sarcoma cells is maintained in the rat, and it was thought that their relative importance would be shown by the following procedure:

A rat, the immunity of which to sarcoma was proved, was exposed to X-rays for a period sufficient to cause the lymphocytes to drop to a few per cent. of their previous numbers, and an inoculation of sarcoma cells was then made.

The experimental arrangements were as follows:—A rat, proved to be immune, was placed in a small wooden box with a thin lid and exposed to X-rays from a Coolidge tube, the anode of the tube being 27 cm. distant. The radiation selected for this purpose had a wave-length ranging from about $2·6$ to $4·2 \times 10^{-9}$ cm.

It was produced under the following conditions: the heating current in the Coolidge tube was 4·3 ampères, and the equivalent spark-gap was 6 cm. between spheres 5 cm. in diameter. The quantity of radiation was measured by allowing the beam of X-rays to enter a small gold leaf electroscope placed 225 cm. from the anode. The readings of the instrument were used as a check on the constancy of the radiation from the Coolidge tube, and the instrument was itself checked by the reading produced by the γ-rays from a standard quantity of radium placed at a fixed distance from the electroscope.

There is no generally accepted method by which the quantity of X-radiation may be specified. Some idea, however, of the quantity of radiation received by the animals in these experiments may be obtained when it is stated that a Sabouraud pastille placed at the centre of the wooden box turned to the standard tint 1 B in 2½ hours ($\frac{1}{3}$ B in 45 minutes, $\frac{4}{5}$ B in 2 hours). This radiation caused the leaf of the electroscope to fall at the rate of about 300 divisions per minute.

Preliminary observation showed that exposing a medium-sized rat for

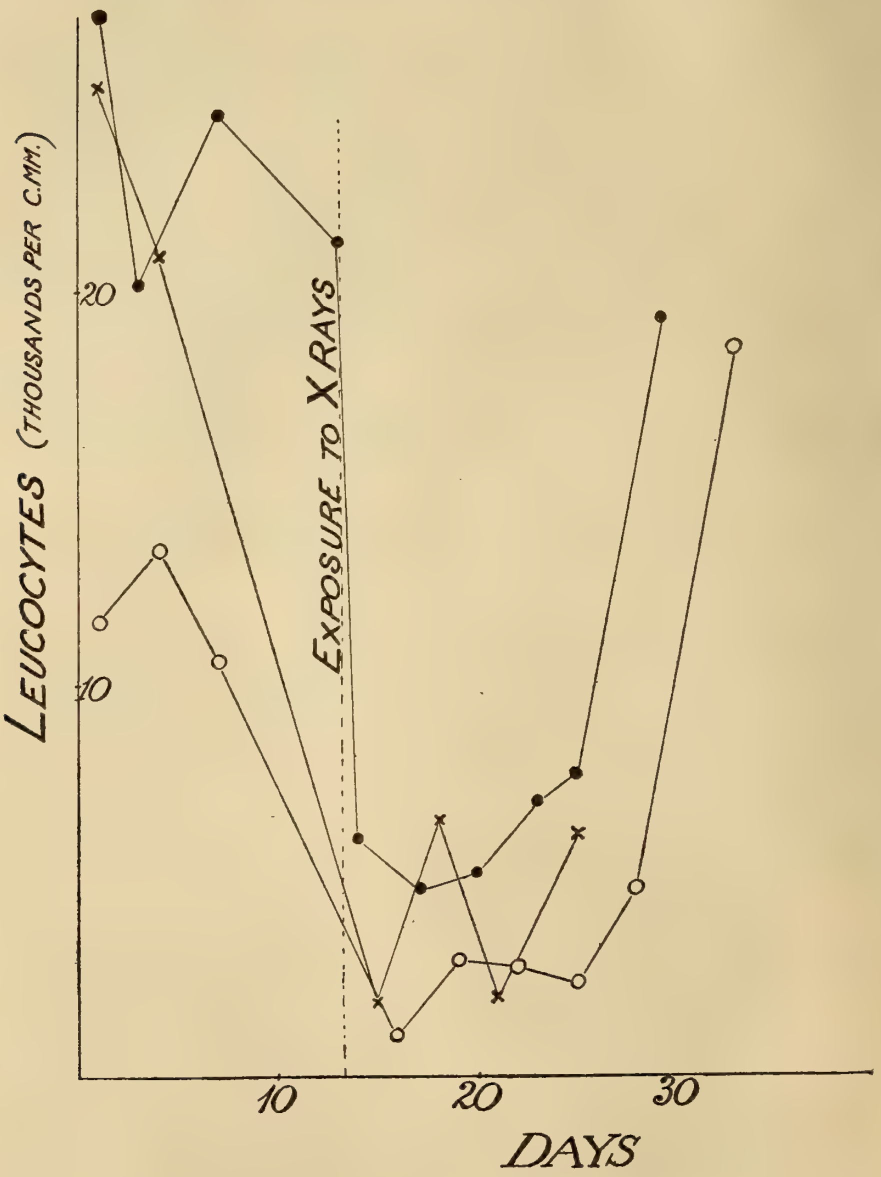

FIG. 8.

½ hour to the radiation under the conditions specified was sufficient to produce well marked changes in its blood; there was, however, a return to a normal content within about 7 days. Prolongation of the exposure to 1 hour leads to a very large reduction in the number of the white cells, so much so that a differential count was hardly practicable. The extent of the destruction of white cells may be gauged from the chart of three rats in fig. 8.

Four rats of proven immunity were irradiated for ¾ hour; the next day they were inoculated with 0·05 c.c. of sarcoma cells, suitable controls being provided. The inoculations into the irradiated animals in no case developed into tumours, but the nodules persisted longer than was the case with ordinary immune rats. Microscopical examination of grafts in the interval 2–6 days after the inoculation showed, however, a distinct contrast to the picture which is typical of a graph of sarcoma cells in an ordinary immune rat (*vide* description, p. 20). The impression was obtained that a little more radiation might lead to a successful growth of sarcoma in the immune animals. Fourteen immune rats were therefore irradiated under the same conditions as before, but for 1½ hours. One or two days after, they were inoculated with sarcoma; the sarcoma cells were found to grow in these animals, which, previous to irradiation, were immune. The chart in fig. 9 shows the gradual development of the tumours. Examination of grafts in these animals showed a condition of growth of the sarcoma which could not be distinguished from that obtaining in normal animals (*vide* figs. 12–14, p. 19).

It will be observed that although growth occurred with the formation of small tumours, there was a great tendency for their subsequent retrogression.

4. *The Effect of Experimental Alteration of the Sarcoma Cell before Inoculation into Susceptible and Immune Animals.*

(a) *The Inoculation of a Mixture of Rat Sarcoma and Rat Spleen.*—The spleens from normal and immune rats were removed and emulsified. A mixture was then made with sarcoma emulsion in varying proportions, which was then inoculated into normal rats.

Illustrations are given of two typical experiments (*vide* figs. 10 and 11). The data in Table IV show the deterrent effect upon growth of the spleen mixtures. Note must be made that when tumour only was inoculated, more sarcoma emulsion was introduced than in the case of the mixtures.

Similar experiments were carried out with liver mixtures; no interference with the growth of the sarcoma was observed.

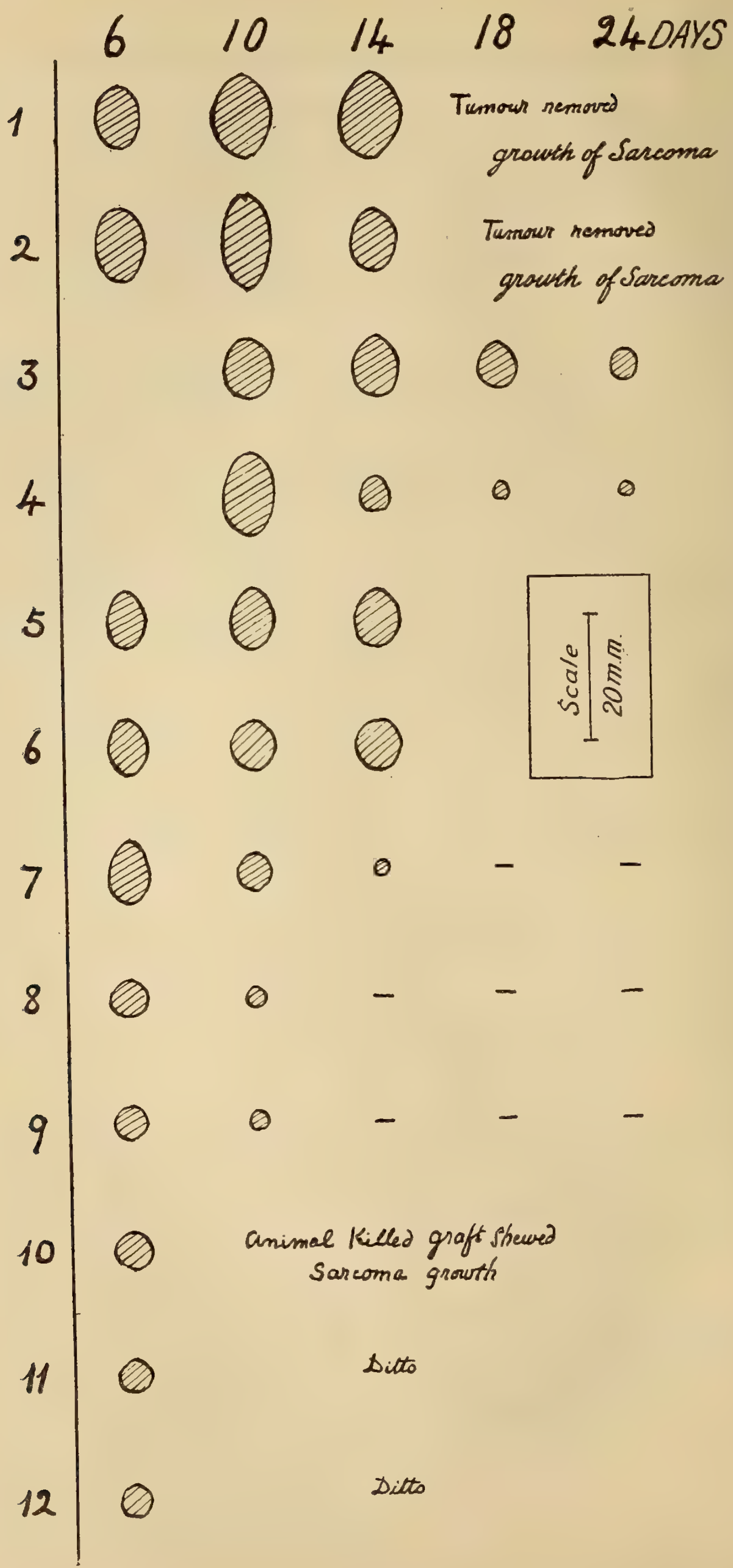
6
10
14
18
24 DAYS
1
Tumour removed growth of Sarcoma
2
Tumour removed growth of Sarcoma
3
4
5
Scale 20 m.m.
6
7
8
9
10
Animal Killed graft shewed Sarcoma growth
11
Ditto
12
Ditto

Fig. 9.

Table IV.

	No. of rats inoculated.	Average superficial area at the 14th day.	Average superficial area at the 21st day.
		sq. mm.	sq. mm.
Tumour alone	33	189	270
Tumour with normal rats' spleen	20	135	186
Tumour with immune rats' spleen	45	63	117

This deterrent effect of the spleen of immunised rats upon tumour growth is not to be attributed to any immunity which might be eventually produced by the spleen cells. This was shown by inoculating tumour on one side of the rat and spleen on the other; no appreciable effect upon tumour growth was observed.

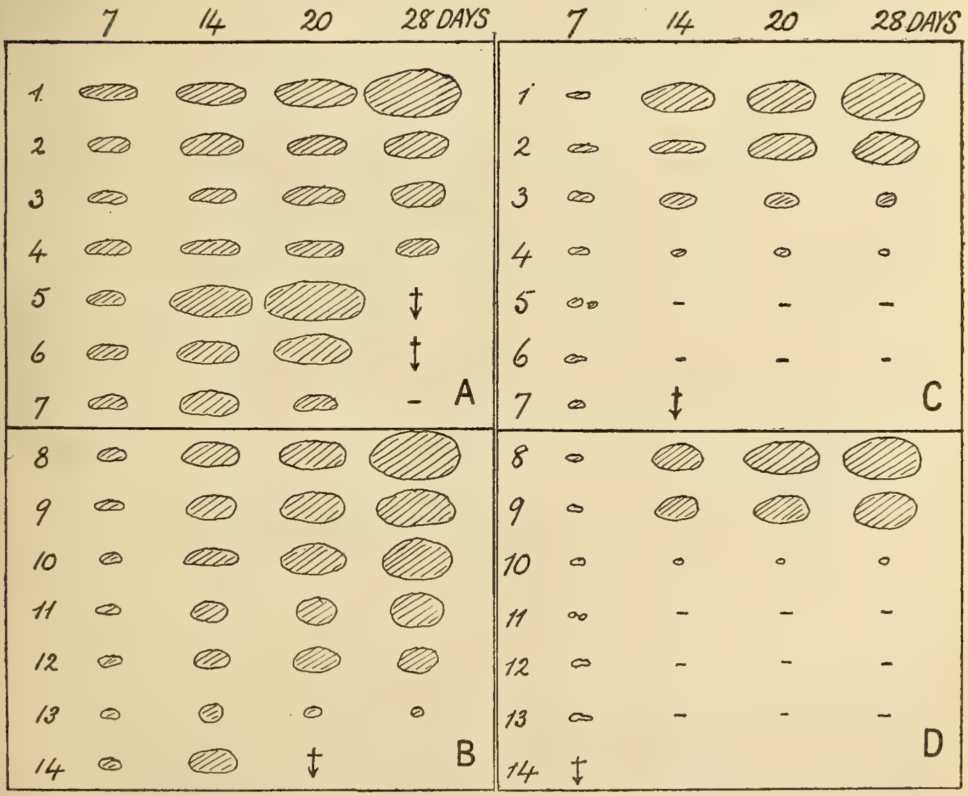

Fig. 10.—A. Tumour only; B. Tumour + Normal spleen; C. and D. Tumour + Immune spleen.

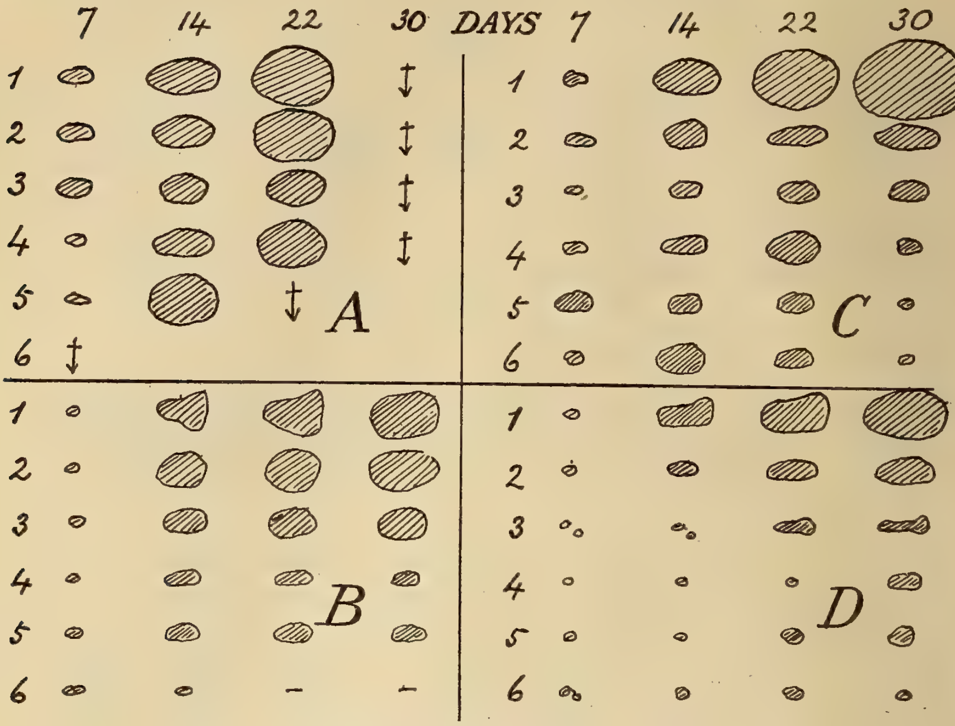

Fig. 11.—A. Tumour only ; B. Tumour + Normal spleen ; C. Tumour + Spleen of tumour-bearing animal ; D. Tumour + Immune spleen.

Tumour Treated with Spleen Extract, Serum, and Plasma of Immune and Normal Rats before Inoculation.

The method of experimentation on these lines was to mix an emulsion of sarcoma cells with either of the above liquids, and after a certain lapse of time to inoculate the emulsion into normal rats.

The spleen extract was prepared by pressing normal and immune spleens in a Buchner's press.

The result of a number of experiments on 102 animals on these lines showed that there was no decided effect upon the subsequent rate of growth of the sarcoma cells.

PART II.—MICROSCOPIC OBSERVATIONS UPON GRAFTS AND TUMOURS.

Side by side with the experiments whose description forms Part I of this paper, microscopic studies have been made of the early stages of growth. These observations upon grafts were carried out in order to determine the reactions of the tissues of the host, and the immediate behaviour of the sarcoma cells, when inoculated under varying experimental conditions.

Material was obtained in the following manner: Rats were inoculated with minced tumour into both axillæ. After varying lengths of time, one graft was removed by operation for histological examination; the other was allowed to remain in order to indicate, by its growth, the degree of susceptibility of the rat. The amount inoculated was 0·05 c.c. The appearances when minute fragments were used were found to be exactly similar to those when 0·05 c.c. was inoculated; in each case the centre of the inoculated mass quickly degenerated, so that, by the second or third day, only cell-débris remained; only the outer surface of the inoculated material for a depth of a few cells remained viable.

Material was fixed in Gilson or Carnoy solution, and stained as a routine with pyronine-methyl green; other methods were also occasionally used, dilute hæmatoxylene (1 in 100 long process), polychrome methylene blue, and azurin 2.

Before describing the microscopic appearances of the grafts, it is necessary to discuss the nomenclature here used with reference to the cells which are found in the connective tissue and reaction tissues of the rat. There can be no confusion about the following cells: fibroblasts, fat cells, mast cells, plasma cells (plasmoidocytes), and polymorphonuclear leucocytes. There remains a polymorphic group of cells, the lymphocytes (polyblasts of Maximow), which have been classified according to their histogenic, hæmatogenic, or endothelial origin, and also according to their characters and functions into adventitial cells, wandering cells, macrophages, clasmatocytes, small amœboid wandering cells, large and small mononuclear cells. Since they appear to grade one into the other as regards both their origin, function, and characters, the term lymphocyte will be here used for the whole group.

1. *The Microscopic Appearances of Subcutaneous Grafts in Susceptible Animals.*

(a) *The Appearance 24 Hours after Inoculation.*—The fatty areolar connective tissue around the inoculated sarcoma emulsion is seen to be distended with a structureless exudate for a distance of from 2 to 4 mm. It is separated from the emulsion by a narrow space or cleft which is often partly filled with collections of polymorphonuclear leucocytes. The groundwork of this reaction tissue consists of oval or round masses (diameter 0·06–0·08 mm.) of hyaline material separated by bands of fine connective tissue fibres. Sparsely scattered over this groundwork, and confined chiefly to the bands between the hyaline masses, are cells of several different kinds (see fig. 12). Close to the emulsion are many polymorphonuclear leucocytes, and immediately outside the crack a few healthy sarcoma cells. At the periphery a small number of mast cells and small fibroblasts are to be seen, as well as fat globules and cells. Lymphocytes form the rest of the cellular elements, they are more or less evenly distributed throughout the tissue, being a little more numerous close to the graft. Lymphatics, capillaries, and small blood-vessels are scattered through the tissue; in many cases their endothelial cells are seen to be dividing (*vide* fig. 12).

The whole of the inoculated sarcoma emulsion presents degenerative changes, except for a few cells deep just within the cleft, where some healthy sarcoma cells are to be seen. Polymorphonuclear leucocytes are everywhere scattered between the sarcoma cells; the groundwork consists of a structureless granular material. The nuclei of the sarcoma cells are shrunken and their chromatin irregularly distributed. The whole nucleus often stains very deeply, whilst the protoplasm is pale and vacuolated.

(b) *Forty-eight Hours after Inoculation* (*vide* fig. 13).—The reaction tissue now measures 1 to 2 mm. in width, being narrower than in the 24-hour specimen. The groundwork consists of a close network of fibrous material, with only small, narrow hyaline masses in its meshes. Through this groundwork the cells have the following arrangement: Near the emulsion, but outside the cleft, are to be seen many healthy sarcoma cells, some dividing; mixed with them are a few polymorphonuclear leucocytes. Lymphocytes are scattered through the tissue; they are more numerous around the blood-vessels than elsewhere, and more numerous than in the 24-hour specimens.

This appearance of greater numbers may be due to their being more concentrated, owing to the shrinkage in width of the reaction tissue. At the periphery of the reaction tissue, the cellular elements are as in the 24-hour specimen, except that a few plasma-cells are to be seen near the outlying blood-vessels. The blood-vessels are more numerous, and are to be seen close to the sarcoma cells, external to the cleft. The sarcoma cells inside the cleft present more advanced degenerative changes than in the 24-hour specimen; only a few healthy cells are to be seen close to the margin.

(c) *Seventy-two Hours after Inoculation* (*vide* fig. 14).—Immediately outside the cleft, closely packed sarcoma cells now form a band of growth encircling the originally inoculated material, which now consists of structureless cell-débris. Among the sarcoma cells are a small number of lymphocytes, numerous blood-vessels, and lymphatics. Outside this band of growth the reaction tissue is similar to that seen in the 48-hour specimen, except that blood-vessels are more numerous and in places closely packed together, while lymphocytes and plasma-cells are less numerous.

(d) *Four to Seven Days after Inoculation.*—The reaction tissue of the host shows no change. As the sarcoma invades the reaction tissue next to it, fresh reaction tissue is formed at a distance. In this way growth proceeds, being always separated from the normal tissues of the host by a thin capsule of vascular fibrous connective tissue.

Summary.—The reaction tissue consists first of inflammatory œdema, which subsequently subsides, and allows the tissue elements to become more closely packed. After two days the tissue consists of a vascular connective tissue, through which are scattered a few lymphocytes. The sarcoma cells within the cleft soon present degenerative changes, and by the third day only cell-débris remains. At the end of 24 hours, healthy sarcoma cells, some dividing, are seen outside the cleft; on and after the

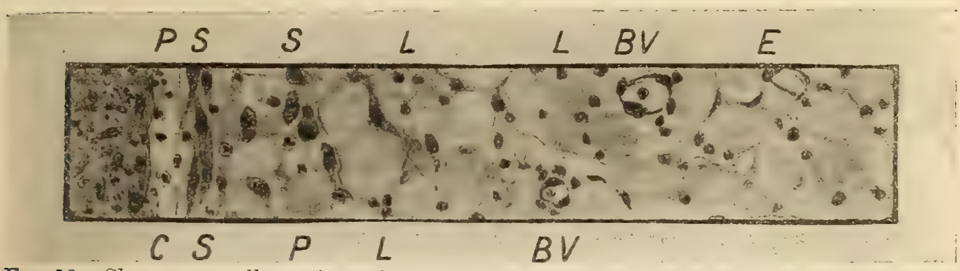

Fig. 12.—Shows a small portion of the margin of the inoculated material, to the left; separated by the "cleft" from a portion of the reaction tissue of the host, to the right; 24 hours after inoculation. S, sarcoma cells outside "cleft"; P, polymorphonuclear leucocytes; BV, blood-vessels; F, fibroblasts; L, lymphocytes; C, cleft.

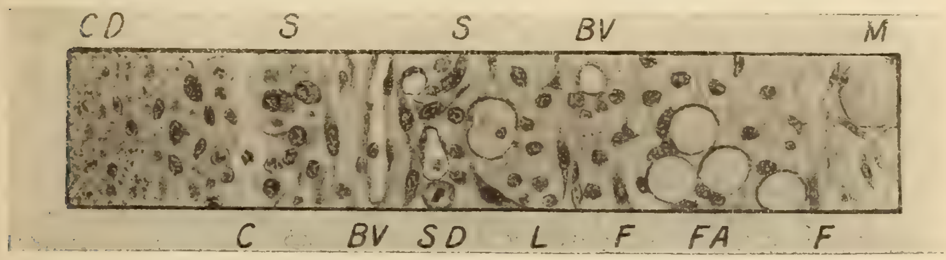

Fig. 13.—Shows the same structures 48 hours after inoculation. F, fibroblast; FA, fat; M, muscle; BV, blood-vessels; L, lymphocyte; S, sarcoma cell; SD, sarcoma cell dividing; CD, cell débris; C, cleft.

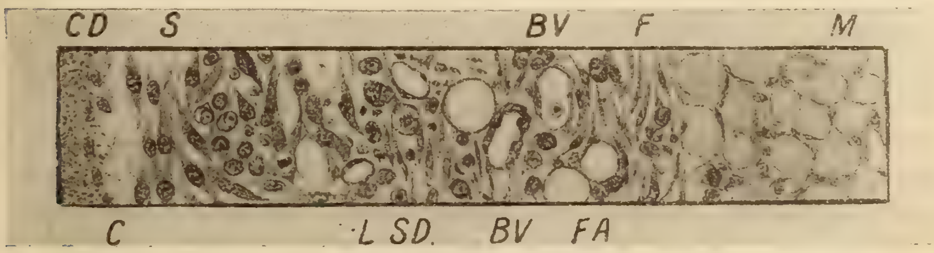

Fig. 14.—Shows same structures 72 hours after inoculation. Letters as above.

third day they here form a definite band of growth. As the sarcoma cells extend out into the reaction tissue, so fresh reaction tissue is formed more distantly.

2. *The Microscopic Appearances of Grafts in Immune Animals.*

For this purpose, animals which had had two previous inoculations, with negative result, were used.

(a) *The Appearances 24 Hours after Inoculation.*—The reaction tissue consists of an inflammatory œdema precisely similar to that seen in susceptible animals. The sarcoma cells also present a similar appearance.

(b) *The Appearances after* 48 *Hours* (*vide* fig. 15).—The groundwork of the reaction tissue is like that seen in susceptible animals; fibroblasts, fat cells, mast cells, blood-vessels, lymphatics, and polymorphonuclear leucocytes have a similar arrangement. In contrast to these similarities, the arrangement of the lymphocytes is strikingly different; they are much more numerous, and are especially abundant just external to the cleft, where they form a solid ring of cells, encircling the inoculated emulsion. At the periphery, plasma cells are to be seen near the blood-vessels. As in susceptible animals, a few sarcoma cells are found outside the cleft; the vast majority, however, present degenerative changes; they are oval or circular in shape, their protoplasm is vacuolated, their nuclei are irregular in shape and contain either no, or very few, fine chromatin granules, and a central large irregular nucleolus. Whereas in the susceptible rat the sarcoma cells external to the cleft are more healthy in appearance than those internal, in the immune rat the reverse is the case.

(c) *The Appearances after* 72 *Hours* (see fig. 16).—The lymphocytes have increased in numbers, so that the solid ring of these cells, just external to the cleft, is somewhat wider than in 48-hour specimens. Towards the outer margin of the reaction tissue, an increased number of fibroblasts are to be seen, and collections of plasma cells around the blood-vessels are numerous. External to the cleft only a few degenerated sarcoma cells are to be found; no dividing sarcoma cells are to be seen. In some cases degenerated sarcoma cells are seen to be embraced by large lymphocytes; in in other cases, nuclei or chromatin fragments are seen in vacuoles in these cells. Degenerated sarcoma cells and chromatin granules contained in vacuoles in large lymphocytes (macrophages), as seen in a graft in an immune animal, but also in all cases where degenerative sarcoma cells are disappearing.

(d) *The Appearances after Four to Six Days.*—The sarcoma cells external to the cleft gradually disappear, so that, by the fifth or sixth day, not a vestige of them remains. The lymphocytes do not further increase in numbers. Fibroblasts become more numerous, and by the sixth day form a close meshwork around the graft, replacing the lymphocytes, which gradually disappear. The collections of plasma cells at the periphery and near the blood-vessels persist, and may even grow larger. By the sixth day the reaction tissue has the appearance of an early fibrosis.

Summary.—Compared to what occurs in susceptible animals, the reaction in immune animals presents two striking differences, namely, the great accumulation of lymphocytes on the second and third day and the rapid degeneration and disappearance of the sarcoma cells external to the cleft. The subsequent fibrosis and collection of plasma cells is also not to be seen in susceptible animals. Apart from these differences, the early inflammatory œdema and the later vascular connective tissue appear precisely similar in both kinds of animals.

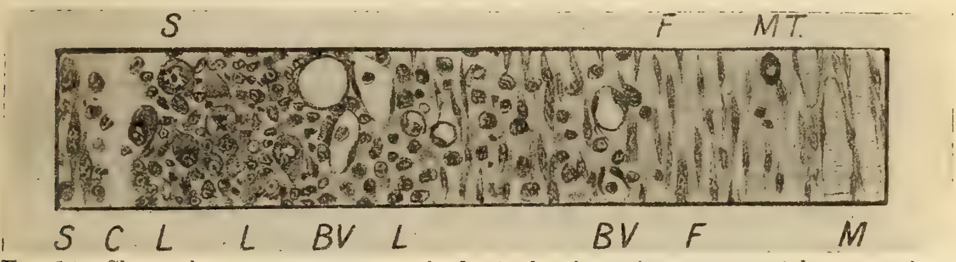

Fig. 15.—Shows the same structures as in fig. 12, but in an immune rat, 48 hours specimen. Mt., mast cell ; M, muscle ; BV blood-vessel ; F, fibroblast ; L, lymphocyte ; S, sarcoma cell ; C, cleft.

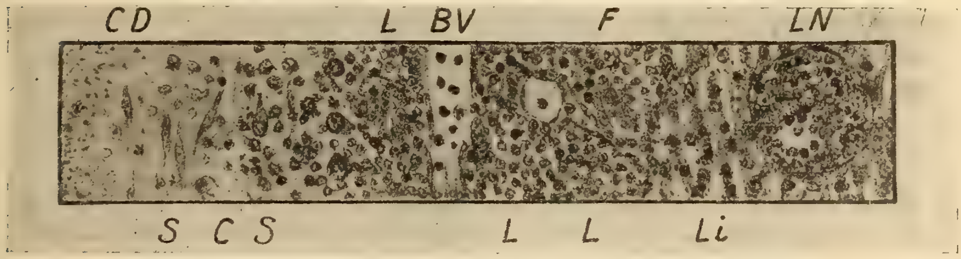

Fig. 16.—Shows the appearances 72 hours after inoculation into the liver of an immune rat. (Subcutaneous inoculation results in a similar appearance.) Li, liver ; LN, lymphoid nodule. Other letters as above.

The Microscopical Appearances when Inoculation is made into Liver and Spleen.—The reaction tissue, both in the case of normal and immune animals, is precisely similar in these organs to that which is produced when inoculation is made into the subcutaneous fatty connective tissue ;

24 hours after inoculation the spleen and liver tissues are found to be everywhere separated from the inoculated material by a zone of inflammatory œdema, and subsequently these tissues remain always separated from the sarcoma cells by the reaction tissue.

In immune animals, on and after the third day, collections of lymphocytes and plasma cells are to be seen in the liver substance, up to a distance of 2·5 mm. from the graft (see fig. 16). In normal rat's liver only a few lymphocytes and plasma cells are to be seen. Similar increase is seen in the spleen when inoculation is there made in the case of immune animals.

3. *The Microscopic Appearances of Grafts in Animals which have been Experimentally Altered before Inoculation.*

(a) *Grafts in Animals the Day After the Removal of the Spleen.*—The appearances of grafts in susceptible animals were found to be similar to those seen in unsplenectomised animals. In immune animals there is a delay in the accumulation of the lymphocytes until the fifth or sixth day, and, during this period, the sarcoma cells form a narrow band of growth outside the cleft; subsequently they die out.

(b) *Grafts in Immune Animals which have been given simultaneously Six or Eight other Inoculations.*—As in splenectomised animals, there is a delay in the accumulation of the lymphocytes until the fourth or fifth day, and a similar temporary growth of sarcoma.

(c) *Grafts in Susceptible and Immune Animals which have been Subjected to X-Radiation before Inoculation.*—Experiments have shown (see fig. 9) that the inoculation of immune animals which have been exposed to X-rays for 1½ hours resulted in the growth of measurable tumours, which persisted for about two weeks. A study of the microscopical appearances of grafts under these conditions showed that the reaction tissue and the behaviour of the sarcoma cells, in both susceptible and immune animals, was exactly similar to that which occurs in susceptible animals. A vascular connective tissue is formed by the host, into which the sarcoma cells wander, divide, and form a band of new growth around the central necrotic area. The growth in such animals appears to be just as vigorous as in susceptible animals, as is shown in fig. 17. After about fourteen days the growth becomes surrounded by, and invaded with, lymphocytes, in a manner similar to that which occurs in disappearing tumours (see p. 24). The tumours finally disappear.

Another set of observations was made with animals exposed to X-rays for half the time, namely, ¾ hour. Measurable tumours did not result, but a temporary growth of sarcoma occurred up to the fifth or sixth day, when accumulation of lymphocytes began, after which the sarcoma cells disappeared.

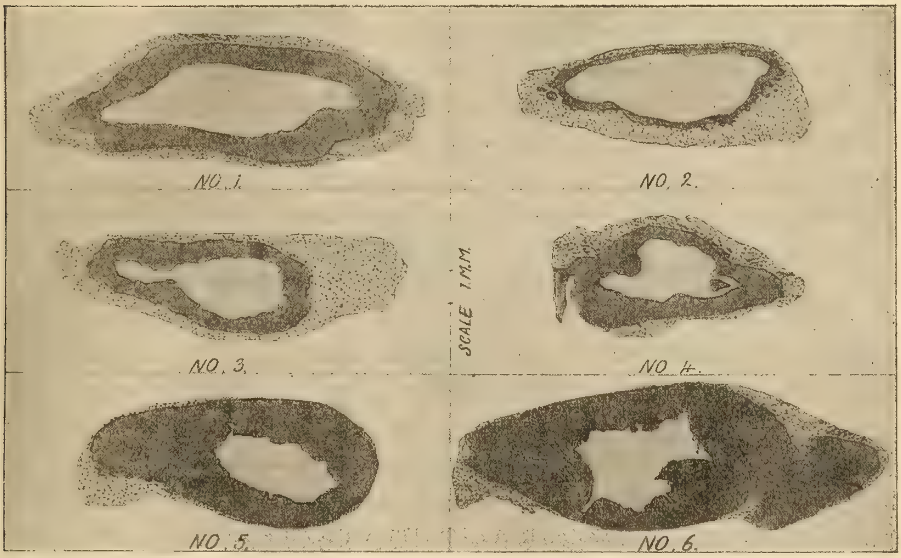

FIG. 17.—Shows tracings made with a projection microscope. Unshaded area—necrotic centre; shaded area—sarcoma growth, except in No. 2, where it represents lymphocytes; dotted area—surrounding connective tissue.

1. 5th day graft in a susceptible animal.
2. 5th day graft in an immune animal.
3. 5th day graft in a susceptible animal exposed to X-rays, 1½ hours.
4. 5th day graft in an immune animal exposed to X-rays, 1½ hours.
5. 10th day graft in a susceptible animal exposed to X-rays, 1½ hours.
6. 12th day graft in an immune animal exposed to X-rays, 1½ hours.

4. *The Microscopic Appearances of Grafts in Animals Inoculated with Sarcoma Cells which have been previously Experimentally Altered.*

Grafts of a Mixture of Sarcoma and Spleen Emulsion Inoculated into Susceptible Animals.—Two series of grafts were studied according as the spleens from susceptible animals or from immune animals were used. Experiments have shown that the inoculation of a mixture of tumour and spleen resulted in delayed growth as compared with the use of tumour alone, and that if the mixture be made with the spleen of immune animals, more interference with growth results than when the spleens from susceptible animals form the mixture (see figs. 10 and 11).

A study of grafts under these conditions shows that very few sarcoma cells are to be seen outside the cleft, until after the fourth or fifth day; during this period, there is some accumulation of lymphocytes in the surrounding connective tissue. Subsequently the lymphocytes decrease in numbers whilst the sarcoma cells multiply and form a tumour.

Grafts of Boiled Sarcoma in Normal Animals.—At the end of 24 hours the surrounding tissues are hardly at all œdematous, and subsequently there is no reaction tissue comparable to that seen when living sarcoma cells are inoculated; there is no accumulation of lymphocytes, fibroblasts make their appearance early and shut off the inoculated material from the tissues of the host by scar tissue.

Grafts of Irradiated Sarcoma in Normal Animals.—Experiments by Chambers and Russ (*loc. cit.*) have shown that if sarcoma emulsion be exposed to a concentration of 0·45 millicurie per cubic centimetre for periods of (*a*) 20 minutes, (*b*) 80 minutes, and (*c*) 24 hours, on inoculation into normal rats, there result (*a*) grafts which grow at a diminished rate, (*b*) grafts which just fail to grow, and (*c*) grafts which present no sign of proliferation.

In the 24-hour irradiated specimens only a few sarcoma cells are seen outside the cleft, no mitosis occurs, degenerative changes begin early and the sarcoma cells soon die out; a moderate amount of lymphocytic infiltration occurs at an early date. In the 80 minutes irradiated grafts considerable proliferation of the sarcoma cells takes place, but later, degenerative changes supervene which are accompanied by an intense accumulation of lymphocytes; the sarcoma cells eventually die out.

The 20 minutes irradiated specimens present proliferation of sarcoma cells; on about the eleventh day some of these appear degenerated, and at this time some lymphocytic infiltration occurs; subsequently the degenerated sarcoma cells and the lymphocytes disappear, whilst the remaining healthy sarcoma cells continue to proliferate in a normal manner.

5. *The Microscopic Appearances of Disappearing and Oscillating Tumours.*

In the connective tissues surrounding these tumours a great accumulation of lymphocytes is to be seen, which contrasts with the relative absence of these cells in progressive tumours.

Lymphocytes are also seen to be mixed with the marginal sarcoma cells, which present decided degenerative changes. It is only towards the centre of such tumours that healthy sarcoma cells are to be seen, in places where there is no lymphocytic invasion. In the surrounding connective tissue, accumulations of plasma cells occur around the blood-vessels, but only a few of these cells are to be seen mixed with the sarcoma cells. The regression of a tumour proceeds from without inwards, and is accompanied by a gradual invasion of lymphocytes; eventually the tumour is replaced by a fibrous connective tissue, containing lymphocytes and collections of plasma cells.

6. *Comparison of Results.*

The facts which these investigations upon grafts have brought out may be conveniently marshalled under (1) the *rôle* of the lymphocyte, (2) the behaviour of the sarcoma cells, and (3) the reaction tissue of the host.

(1) *The Rôle of the Lymphocyte.*

In susceptible animals they are seen in the reaction tissue in small numbers, making their appearance on the second or third day.

In immune animals the reaction tissue is loaded with these cells from the second and third day until the final disappearance of the sarcoma cells.

Both these characteristic reactions are affected by alteration of the experimental conditions along two distinct lines, namely, by changing the condition of the sarcoma cell before inoculation, or by altering the rat's condition before inoculation.

(a) *Changing the Condition of the Sarcoma Cell.*—If the cell be killed by heat before inoculation, no lymphocytic accumulation occurs in either susceptible or immune animals.

If the sarcoma cell be given an exposure to the β- and γ-rays from radium sufficient to prevent any proliferation, some lymphocytes accumulate in the reaction tissue on the second, third, and fourth days.

If the dose of radiation be just sufficient to prevent the growth of a tumour, then in susceptible animals there is a great accumulation of lymphocytes from the fifth day until the final disappearance of the sarcoma cells.

If the dose be sufficient only to delay growth, then an accumulation of lymphocytes occurs about the fifth day, but subsequently they disappear and the growth of sarcoma proceeds as under normal conditions.

If the sarcoma cells be mixed with spleen cells before inoculation, more lymphocytes are found in the reaction tissue than when unmixed sarcoma cells are inoculated into susceptible animals; but they are less numerous than in immune animals.

(b) *Altering the Rat's Condition before Inoculation.*—If rats be splenectomised before inoculation, then in susceptible animals no differences were seen. In immune splenectomised rats there is a decided delay in the onset of lymphocytic accumulation; it is not until the fifth or sixth day that they are present in numbers comparable to that seen in unsplenectomised immune animals on the second or third days.

If immune rats be given six or eight inoculations of 0·05 c.c. instead of two, then a similar delay occurs in the accumulation of the lymphocytes.

If rats before inoculation be given a dose of X-rays sufficient to reduce the number of white cells in the blood by about 90 per cent., then in immune animals the accumulation of lymphocytes is delayed for as long as two weeks. (A smaller dose of X-rays resulted in less delay.)

The accumulation of lymphocytes which occurs around the sarcoma cells, which by then have grown to a measurable tumour, is precisely similar to what is to be seen when tumours are either disappearing or oscillating.

(2) *The Behaviour of the Sarcoma Cells.*

Before attempting to correlate these facts, it is necessary to consider what has been seen to occur as regards the behaviour of the sarcoma cells under these same experimental conditions. In order that these facts may be displayed side by side, Table V has been prepared; the conditions in disappearing, oscillating, and progressing tumours have been subjoined. Detailed descriptions of the behaviour of the sarcoma cells have already been given, and do not require to be again referred to in the text.

On referring to Table V, it can be seen that, in susceptible animals, lymphocytic accumulation only occurs when injured sarcoma cells are inoculated; and that where uninjured or dead cells are used, very slight lymphocytic accumulation is found. Further, it can be seen that when great accumulation occurs the animal is likely to be subsequently immune.

In immune animals a delay in the accumulation of lymphocytes is seen to be associated with a temporary growth of the sarcoma; but that, later, when accumulation of the lymphocytes takes place, the sarcoma cells die out, and the animals remain unsusceptible.

A similar correlation has been seen to hold in the case of tumours. Animals bearing progressive tumours are usually susceptible, they do not present accumulation of lymphocytes in the surrounding connective tissue; the sarcoma cells are in active division. On the other hand, animals bearing disappearing or oscillating tumours are almost invariably immune, they present great accumulation of lymphocytes; the sarcoma cells are either degenerated or not in active division.

(3) *The Reaction Tissue of the Host.*

Apart from the differences already noted, the local reaction of the tissues of the host appear to be the same in susceptible and immune animals. An inflammatory œdema, lasting for 24 hours, is followed by the laying down of a vascular connective tissue. It is important to note that the formation of blood-vessels in the tissues occurs at the same time, and to the same extent, in immune as in susceptible animals; and that the failure of growth in immune animals cannot therefore be accounted for by the failure of a sufficient vascular supply.

Table V.

			The animal altered before inoculation.				The sarcoma cells altered before inoculation.				
		No alteration before inoculation.	By splenectomy.	By a large inoculation.	By exposure to X-rays for $1\frac{1}{2}$ hours.	By exposure to X-rays for $\frac{3}{4}$ hour.	Killed by exposure to heat.	By exposure to radium sufficient to prevent the division of cells.	By exposure to radium sufficient to prevent the formation of a tumour.	By exposure to radium sufficient to produce delay of growth of a tumour.	By admixture with spleen cells before inoculation.
In susceptible animals.	The *rôle* of the lymphocyte.	Very few lymphocytes in the reaction tissue.	As in unaltered animals.				Very few lymphocytes in reaction tissue.	Some accumulation of lymphocytes 3rd–4th day.	Great accumulation of lymphocytes on about the 5th day.	Some accumulation 5th–6th day, but subsequently disappear.	Some accumulation of lymphocytes 3rd–4th day.
	The behaviour of the sarcoma cell.	Sarcoma cells multiply and form a tumour.					No growth of sarcoma cells; none outside the "cleft."	No division of sarcoma cells; some outside the cleft.	Some division of sarcoma cells outside the cleft, but subsequently disappear.	Slow division of sarcoma cells and production of a tumour.	Delayed growth of sarcoma, but production of a tumour.
	The subsequent susceptibility of the animal.	The animal may be either susceptible or immune.	Experiments not carried out.				Animal remains susceptible.	Animal remains susceptible.	Animals usually immune.	May be either susceptible or immune.	May be either susceptible or immune.
In immune animals.	The *rôle* of the lymphocyte.	Great accumulation of lymphocytes 2nd or 3rd day.	Accumulation of lymphocytes delayed to 5th or 6th day.	Accumulation delayed to 4th or 5th day.	Accumulation delayed to about 14th day.	Accumulation delayed to 5th or 6th day.	Experiments have not been carried out.				
	The behaviour of the sarcoma cell.	A few sarcoma cells outside the "cleft"; none divide.	Division of sarcoma cells outside cleft up to 5th day; subsequently die out.	Division of sarcoma cells outside cleft up to 4th day; subsequently die out.	Sarcoma cells divide and produce a measurable tumour which subsequently regresses.	Divisions of sarcoma cells outside the cleft up to 5th day; subsequently die out.					
	The subsequent susceptibility of the animal.	Animal remains immune.	Animal remains immune.	Animal remains immune.	Animal remains immune (few experiments only).	Animal remains immune (few experiments only).					

PART III.—GENERAL DISCUSSION AND CONCLUSIONS.

Preparatory to a brief discussion on the trend of our observations, we give below some of the main facts which have been ascertained :—

1. Jensen's rat sarcoma almost invariably grows when inoculated into rats.

2. Once having been inoculated, the rats, in over 90 per cent. of the cases, are immune to a second inoculation. The different phases of growth which the tumours exhibit are intimately bound up with the varying degree of immunity set up by the animal.

3. When a rat is immune to the inoculation of the sarcoma cells, the spleen of the animal generally shows a high content of lymphocytes and plasma cells.

4. Mixture of the spleen with the sarcoma cells before inoculation causes a retardation of growth in the resulting tumour ; this is more marked with the spleen of an immune animal than with that of a normal one.

5. Sarcoma cells may remain as long as three days in an immune rat, and then be successfully re-inoculated ; this period corresponds with the interval required for the accumulation of lymphocytes around the graft.

6. The essential difference in the processes initiated on introducing sarcoma cells into normal and immune rats consists in a marked accumulation of lymphocytes around the graft in the immune rat.

7. By damaging the sarcoma cells by irradiation, the subsequent reaction of the normal animal resembles that of the immune one.

8. By damaging the rat, the accumulation of lymphocytes is delayed and growth of the sarcoma occurs.

9. Rats may be made immune by inoculation of sarcoma cells which have previously been exposed to the β- and γ-rays from radium.

10. By exposure to X-rays, an immune rat may be converted into a tumour-bearing animal.

The majority of normal rats are susceptible; in a few cases only, small nodules follow inoculation. In all such cases, when microscopical examination of the early stages of grafts has been made, some proliferation of the sarcoma cells has been observed. The complete inhibition of proliferation, which occurs in immune animals, has not been observed in normal animals. A complete natural immunity, comparable with acquired immunity, has not been met with.

The microscopical appearances of a graft whose growth is being controlled at an early stage is similar to what is seen in disappearing tumours; what is termed natural immunity appears to be an immunity acquired during the regression of a small nodule.

Further, as will be discussed later, acquired immunity appears to depend on a process similar to that which occurs in disappearing tumours, but in this case the control of growth begins at once, so that no proliferation of the sarcoma cells occurs.

After inoculation, the majority of rats subsequently become immune; in some the immune condition appears soon after inoculation, in which case the tumour resulting from the first inoculation only reaches a small size; if the onset of immunity be further delayed, the tumour may reach a large size before regressing.

The tumour may never regress, but only the rate of growth become less, when the rat is found to be nearly always unsusceptible.

It follows that the onset of unsusceptibility, and the power to inhibit the growth of an established tumour, are closely associated.

It has, however, been seen that an animal bearing a progressively growing tumour, in which, therefore, no measurable inhibition of growth is occurring, may be either susceptible or immune. This might be taken as evidence that the two processes are not identical; on the other hand, a force which is sufficient to kill a few sarcoma cells struggling to establish themselves may have no measurable effect upon a large, well-established tumour. An alternative explanation has been given, on the view that a concomitant immune condition is produced by the re-inoculation itself, which is sufficient to prevent its taking, but insufficient to affect the progressive tumour. As will be seen later, there is considerable evidence that the two conditions—unsusceptibility and the power to inhibit growth of an established tumour—are identical (see p. 30).

Both of the conditions, susceptibility and immunity, are generalised; no matter where inoculation be made, the graft will take or fail to grow, as the case may be.

Immunity depends upon the ability of the animal to prevent the growth of, and to destroy, sarcoma cells when introduced into its body. This condition has been found to be associated with changes in the spleen, in respect of the number of lymphocytes and plasma cells present; nevertheless, it has not been possible to define exactly the relation between these factors. There is, however, other evidence that the spleen plays some part, viz.: (1) When mixtures of spleen and tumour are inoculated, the use of immune spleen causes a greater delay in growth than in the case of normal spleen. (2) If immune animals be splenectomised, the microscopic study of grafts shows that some growth of the sarcoma occurs. (3) If immune animals be splenectomised, and at the same time a large dose of sarcoma be given, in a few cases measurable tumours result. (4) If immune animals be exposed to X-rays before inoculation, measurable tumours result, and the study of grafts shows that considerable growth of sarcoma occurs; at the same time, profound changes take place in the spleen; lymphocytes, and, to a less extent, plasma cells, are completely destroyed.

Our observations as to the effects of X-rays upon the immune condition are confirmatory of the experiments on similar lines initiated by Murphy and Morton (5), who showed that mice immunised with defribrinated blood could be rendered susceptible to mouse carcinoma by a suitable exposure to X-rays.

Murphy (6) has also shown that the chick embryo, up to the 18th day, will support the growth of rat sarcoma, but that if, during this period, adult chicken spleen or bone marrow be inoculated, then the established rat sarcoma will be destroyed. It was found that this destruction was associated with an accumulation of lymphocytes in the connective tissue around the sarcoma.

Other evidence that the lymphocytes, and less certainly plasma cells, are an important factor in the condition of immunity is, however, forthcoming. The evidence of grafts study is very strong, and it does not appear necessary to repeat this again; the reader is here referred to Part II. (1) The *Rôle* of the Lymphocyte, and also to the observations of da Fano (*loc. cit.*).

Reference may be made again to the fact that sarcoma cells are able to survive in an immune rat for three days, and that this is the period at which the local accumulation of lymphocytes around the inoculated material reaches its maximum. Finally, it may be pointed out that, just as the destruction of sarcoma cells, when introduced into an immune rat, is always associated with a local accumulation of lymphocytes, so when the growth of an established tumour begins to be controlled, there is likewise a great accumulation of lymphocytes in the immediate neighbourhood of the tumour.

It may therefore be concluded that lymphocytes play an important part in the process by means of which an animal is able to destroy sarcoma cells, and that their local presence is necessary for this destruction, and that in their absence locally the sarcoma cells will proliferate.

We are, as yet, entirely ignorant of the mechanism by means of which the lymphocyte is brought to the sarcoma cell. The facts indicate that the lymphocyte does not merely act as a scavenger of sarcoma cells, killed, for instance, by some toxin; for if this were the case, it must be assumed that such actions as splenectomy and X-ray exposure destroy the toxin as well as the lymphocyte, unless the toxin is secreted by the lymphocyte, which is a possibility to be taken into account. The exact time relations between lymphocyte accumulation and sarcoma degeneration have not, as yet, been thoroughly worked out; but from the study of grafts, the degeneration is roughly concomitant with the local accumulation of the lymphocytes. It is certain that the lymphocytes do not ingest the sarcoma cells until some degeneration has taken place in the latter.

The negative evidence (*vide* p. 16) in the search for a toxin in the circulating fluids indicates that it may be manufactured locally by the lymphocyte. There is also more direct evidence in that where for any reason there is a delay in the local accumulation of lymphocytes, then the sarcoma cells instead of degenerating remain healthy and proliferate even in an immune animal, and it is not until accumulation occurs that growth is controlled. Degenerated sarcoma cells in the absence of lymphocytic infiltration have never been observed, except in the necrotic centre of tumours, where the degenerative changes are different and where lymphocytic infiltration is not seen. It would appear for this reason also that lymphocytic infiltration is not a reaction to dying or dead sarcoma cells, or else it would occur under such conditions.

In favour of there being an association between the production of immunity and the local accumulation of lymphocytes is the fact that in all tumours of the disappearing and oscillating types great accumulation of lymphocytes occurs, and this is associated with unsusceptibility. It would appear therefore that without local accumulation of lymphocytes neither graft destruction, tumour destruction, nor production of immunity can occur. Finally, it may be again mentioned that the condition of immunity to the graft has not in any respect been observed to be due to a failure on the part of the host to supply the necessary connective tissue or blood supply; but all the evidence goes to show that the immunity consists of a positive action on the part of the host.

The above statement refers only to Jensen's rat sarcoma, for in the case of mouse carcinoma Russell (7) has shown that the failure of an inoculation in an immune animal is to be ascribed to a failure on the part of the host to supply the "specific stroma reaction."

The Bearing of the Observations upon Malignant Disease in the Human Subject.

Spontaneous disappearance of malignant disease in the human subject is very rare; there is, however, no reason to doubt the clinical observation that occasionally growths, malignant in all their aspects, do spontaneously disappear. Clinical study has also shown that in some cases, although the tumour may not disappear, its growth is not continuously progressive, but that sometimes growth is held in check or may even regress, though finally proving fatal. There is, in fact, ample evidence to show that an active resistance to the growth of the tumour is in some cases set up in the human subject.

The bearing of our observations upon malignant disease in the human subject is of a dual nature. If the *rôle* which the lymphocyte plays in immunity towards Jensen's rat sarcoma proves to apply also to the human malignant growths, then the lines along which successful treatment may possibly be attained are to some extent indicated. In the first place, it may be possible by stimulating the growth of lymphoid tissues to produce a concentration of these cells at the locality of the growth. This might be accomplished by the inoculation of malignant cells rendered harmless by irradiation or of some other material.

In the second place, reference may be made to the treatment of malignant disease by means of X-rays or the rays from radium (β- and γ-rays). The aim of radiotherapy in malignant disease is to cause the destruction of the malignant cells with a minimum amount of damage to the adjacent normal tissues. Under many conditions of irradiation of the human subject, and especially in the treatment of deep-seated malignant growths, it happens that the normal tissues are subject to a very considerable degree of irradiation.

In view of the fact that the lymphocytes play an important part in the healing of growths of Jensen's rat sarcoma, and because these cells have been shown to be especially vulnerable to these radiations, it follows that particular care should be taken in this treatment of cancer in the human subject to protect the rest of the body from radiation, more especially those parts where lymphocytes occur, namely, the blood, lymph, bone marrow, lymphatic glands, and spleen.

It is known (8) that a small dose of rays may stimulate the production of some of the cellular constituents of the blood. With prolonged irradiation, however, the probability of destroying cells which are capable of resisting the growth of the invading cancer cells suggests the advisability of taking steps to avoid the irradiation of any but the malignant tissues, and, if possible, to restrict the blood supply of all these tissues, malignant or otherwise, through which the radiation penetrates.

The expenses of this research were partly defrayed by a grant to one of us (S. R.) from the Royal Society.

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The Germicidal Action of Ultra-Violet Radiation, and its Correlation with Selective Absorption.

By C. H. Browning, M.D., D.P.H., Director of the Bland-Sutton Institute of Pathology, Middlesex Hospital, and Sidney Russ, D.Sc., Physicist to the Middlesex Hospital.

(Communicated by Prof. A. W. Porter, F.R.S. Received February 27, 1917.)

[Plate 3.]

A new method is here described which enables us to say definitely what portion of the ultra-violet spectrum is especially effective in germicidal action and the wave-length of the radiation at which such action practically ceases. Briefly the method consists of inoculating a gelatine* plate with micro-organisms instead of sensitising it with a silver salt. We find that when a spectrum is formed on this it produces what may be called an image, where germicidal action occurs, and this image may be rendered visible by a process of incubation, which encourages a copious growth of those organisms which have not been affected by the radiation, whereas the affected parts remain practically transparent. Such an exposed and incubated plate can be used as an ordinary negative for producing positive contact prints or, equally well, may be photographed by light reflected and scattered from the bacterial surface.

The study of the action of radiation, visible and otherwise, upon micro-organisms is not a new one. The period 1894–6, associated with the work of Marshall Ward,* Wesbrook,† and D'Arcy and Hardy,‡ conferred upon such investigations an exactness which had been previously entirely lacking, both as regards knowledge of the most effective germicidal portion of the visible rays, and also with regard to the essential chemical processes associated with such action.

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* For convenience, an agar plate was used in most of the experiments.

The present investigation deals with one particular region of ultra-violet radiation, which we find has much greater germicidal action than any part of the visible spectrum, and an attempt is made to correlate such germicidal action with the physical phenomenon of selective absorption.

The quartz spectrometer (Design C), made by Messrs. Hilger, is so constructed that photographs may be obtained ranging from wave-lengths 7000–2100 Å.U.;§ this region of the spectrum is spread over a length of about 19 cm. (it varies slightly with each instrument).

The radiation emitted by an arc of pure tungsten, when focussed on the slit of the instrument and received on the photographic plate, is seen to consist of a series of bright lines very closely packed together extending as far as 2100 Å.U. Thus it is a very suitable source of intense ultra-violet radiation over a wide range.

In order to determine what part of this radiation exerts germicidal action, the following procedure was employed: A glass plate, similar to the photographic plate used in this instrument, was coated with a thin layer of nutrient agar; then over this surface a thin layer of a living bacterial emulsion (*Staphylococcus pyogenes aureus*) was painted. The plate thus inoculated was then placed in the carrier of the instrument, and the shutter opened to allow the radiation from the arc to fall upon a narrow strip of the bacterial film. After an appropriate exposure the plate was removed from the spectrometer, and then incubated at 37° C. for 48 hours in order to see, by the resulting growth, what effect had been produced upon the organisms by the various constituents of the beam.

Fig. 1 shows the germicidal effect of the rays upon *Staphylococcus pyogenes aureus* for three different times of exposure of the organisms, viz., 6, 12, and 24 minutes. The radiation utilised, as shown by its action on a photographic plate, extended right across the bacterial film; the germicidal action is, however, restricted to a region which ranges from a wave-length of 2940 to about 2380 Å.U. The black lines indicate the bactericidal action. The illustration was obtained by photographing the bacterial film by means of transmitted light. From this negative a transparency was made, and this was employed for the print.

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* Marshall Ward, 'Phil. Trans.,' B, 1894, p. 961.

† Wesbrook, 'Journ. Path. and Bact.,' vol. 3, p. 70 (1896).

‡ D'Arcy and Hardy, 'Journ. Physiology,' vol. 17, No. 5, p. 390 (1894).

§ Å.U. signifies Ångström units, the standard by which wave-lengths are measured.

Photographs taken with long exposures always show the presence of scattered radiation, and it is interesting to observe that the organisms appear to be stimulated in their growth in the region just between two portions which have been exposed to the full radiation. The increase of bacterial growth was confirmed microscopically, but it is not necessarily due to the scattered radiation. This stimulation is only seen satisfactorily in the original.

The effect of prolonging the exposure from 6 to 24 minutes is to increase somewhat the range of lethal action, but only to a comparatively slight extent. A more prolonged exposure brings out prominently the region of the spectrum at which germicidal action practically stops. In order to increase the amount of radiation reaching the bacterial film, the slit was widened; this had the effect of causing the lines of the spectrum to overlap sufficiently to form a practically continuous band of radiation.

The procedure followed was otherwise identical with that which has just been described; the bacterial film was first exposed to the whole range of spectral lines, then incubated, to see what effect had been produced upon the organisms by the various constituents of the beam.

The central strip of fig. 2 shows the result of a prolonged exposure (about 3½ hours) of the organisms to the tungsten radiation. It will be seen that germicidal action, indicated by the black region, occurs throughout a region of wave-lengths extending from about 2960 to 2150 Å.U. The most striking feature is the sharp line of demarcation occurring in the region of the former wave-length. Ether vibrations of wave-length 2960 Å.U. have a marked germicidal action; an increase of 1 or 2 per per cent. in this wave-length is, practically speaking, sufficient to bring one to a region of the spectrum devoid of such germicidal action.

A control experiment was made to decide whether the radiation had any effect upon the agar. A portion of it was irradiated, and then inoculated with a suspension of an agar culture of *Staphylococcus pyogenes aureus*, which had been washed three times by centrifuging with sterile 0·8 per cent. sodium chloride solution. No difference was detected in the density of growth of the organisms over the irradiated and the non-irradiated portions of the agar.

The existence of this sharp line of demarcation strongly suggested that selective absorption was playing some part. This was put to the test by examining the absorption spectrum of a suspension of the organisms which were used in the experiment just described. A small quartz vessel was filled with the bacterial emulsion and placed in front of the slit of the spectrometer; a series of photographs was then taken of the radiation transmitted through the emulsion. The bottom strip (fig. 2) shows that the region of wave-lengths absorbed by the bacterial emulsion corresponds almost exactly with that portion of the spectrum which has marked germicidal action. We shall return to this point later on.

The Germicidal Action upon Various Organisms.

The method which has been described to determine what portion of the ultra-violet spectrum is responsible for germicidal action is well adapted for comparative tests of the effects of these rays upon different organisms.

(a) *Bacillus coli communis and Bacillus typhosus.*—To determine the respective ranges of susceptibility of these organisms, emulsions from agar cultures were made and spread over a surface of agar, which took the place of the photographic film, as already described. A central strip, about 3 cm. wide, running the whole length of the plate, was separated from the rest of the agar by two glass strips a few millimetres in width. The central strip was painted over with the emulsion of one of the organisms, and the rest of the agar with that of the other organism. The plate was then placed in position in the camera, and the length of the slit adjusted so that the radiation from the arc illuminated both surfaces over which the organisms were spread, one of the glass strips lying exactly along the middle of the exposed area. In this way the two organisms were exposed to radiation of identical character for the same time; after a suitable exposure, the camera attachment was racked down, so that the second glass strip was in the centre of the exposed area, and another exposure made. The agar plate was removed and incubated overnight; the resulting growth on the plate showed which organism had been affected by a greater range of the rays.

A series of observations, ranging from 5–10–20–30 minutes, showed that a lethal effect upon *B. typhosus* was obtained over a wider range than with *B. coli;* this difference in range of susceptibility was small, but quite definite for each of the exposures: for an exposure of five minutes, a lethal effect was obtained on the *B. coli* over a range of wave-lengths 2960–2450 Å.U.; for the *B. typhosus* the range was 2960–2400 Å.U.; when the exposure was lengthened to 30 minutes, the lethal effect on *B. coli* ranged from 2960 to 2200 Å.U.; and that of the *B. typhosus* from 3000 to 2100 Å.U. Such small differences could hardly serve as a sure method of differentiation of these organisms.

(b) *Acid-fast Bacillus* (*Timothy Grass B*) *and the Meningococcus.*—The ranges of susceptibility of these organisms to the rays in question was compared directly with staphylococcus by the method detailed above. (In the experiments in which the meningococcus was compared, "trypagar" was employed as the medium.) After suitable exposures the agar plates were incubated, and the range of wave-lengths over which a lethal action was subsequently observed was measured for the different organisms. The meningococcus was found to be susceptible over a slightly greater range than the staphylococcus; exposure of these two organisms to the same radiation for the same time (20 minutes) resulted in a lethal action upon the meningococcus over a range 2960–2240, the range for staphylococcus being 2960–2320 Å.U.

In the case of the acid-fast bacillus, the result was reversed, the staphylococcus being slightly more susceptible than the acid-fast bacillus.

The Connection between Germicidal Action and Selective Absorption.

The photograph which serves to illustrate the selective absorption of the bacterial emulsion was obtained with an exposure of two minutes. A single observation is obviously not sufficient to prove that the region beyond a wave-length of 2960 Å.U. is selectively absorbed. Therefore a series of photographs was taken in which the exposure ranged from five seconds to five minutes, and the fact that practically identical records resulted, except in the respective densities of the spectral lines transmitted through the bacterial emulsion, may be taken as evidence that wave-lengths shorter than 2960 Å.U. are actually absorbed in a selective manner. By increasing the exposures to as much as 20 minutes, only a few additional lines made their appearance beyond the line of demarcation in question.

It seems therefore that the conclusion may justifiably be drawn that ultra-violet radiation between wave-lengths 2960 and 2100 Å.U. is germicidal to bacteria, and that rays over this range of wave-length are also particularly absorbed by the substances of which such bacteria are composed. We have found experimentally that such substances as human serum and egg albumen also have a well marked absorption band for wave-lengths ranging from 3180 to 2100 Å.U. (and also possibly beyond, but this we have not investigated). If further we recall the fact that human skin in a layer as thin as 1/10 mm. is practically opaque to radiation over a very similar range of wave-lengths, then we may look upon this region as one for which protoplasm has a particular power of absorption. The high degree of correlation between the germicidal action of a particular portion of the ultra-violet radiation with an enhanced degree of absorption of such radiation by the organisms, which appears to have been clearly established, does not, of course, explain the bactericidal effect, but the enquiry now takes on a physico-chemical aspect as well as a purely biological one. Attention may be called to the correspondence between our result and that found by Grotthus in 1818* for photo-chemical action, which he expressed in the law "that only those rays which are absorbed can produce chemical change."

The Bearing of the Observations on the Clinical Uses of Ultra-Violet Radiation.

The preceding data indicate that, from the clinical point of view, there are two distinct regions of ultra-violet radiation :—

Group 1.—A portion which begins where vision fails, namely 3800, and extends to 2960 Å.U. These rays have no marked germicidal action ; they are capable, however, of penetrating a considerable thickness of human skin.

Group 2.—A portion which extends from 2960 to nearly 2100 Å.U. These rays have very marked germicidal action, the region of maximum effectiveness being between 2800 and 2540 Å.U. The penetrating power of these rays is, however, very small; they are completely absorbed by as little as 1/10 mm. of human skin.

It remains to consider to what degree these two regions of the ultra-violet radiation contribute to the beneficial effects resulting from the clinical use of these rays.

If a powerful source of ultra-violet radiation be directed upon an infected wound, the result of an adequate exposure will be that the pathogenic organisms on the surface will be directly killed ; it is, of course, not determined what action these particular rays may themselves exert on the protective mechanism of the living tissues. Organisms at a depth cannot be killed directly, for the germicidal rays do not reach them. The passage through the tissues of rays constituting Group I, together with the luminous portion of the rays, may not, however, be without effect upon them. It is well to bear in mind the possibility of the luminous rays having definite physiological effects, for it must be remembered that such rays penetrate to a greater depth of the tissues than does even Group I of the ultra-violet rays.

It has been stated by several writers that deep-seated conditions have been benefited by exposure to some strong source of visible and ultra-violet radiation. It remains to be found to what extent the different parts of these two octaves of radiation are concerned in such clinical results, although, as has been already stated, there is no evidence that deeply situated organisms can be affected by those ultra-violet radiations which are known to possess powerful bactericidal action.

We have pleasure in recording our thanks to the British Thomson-Houston Company for their gift of the tungsten used in this investigation.

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* See Gilbert's 'Ann. d. Phys.,' vol. 61, p. 50 (1819).

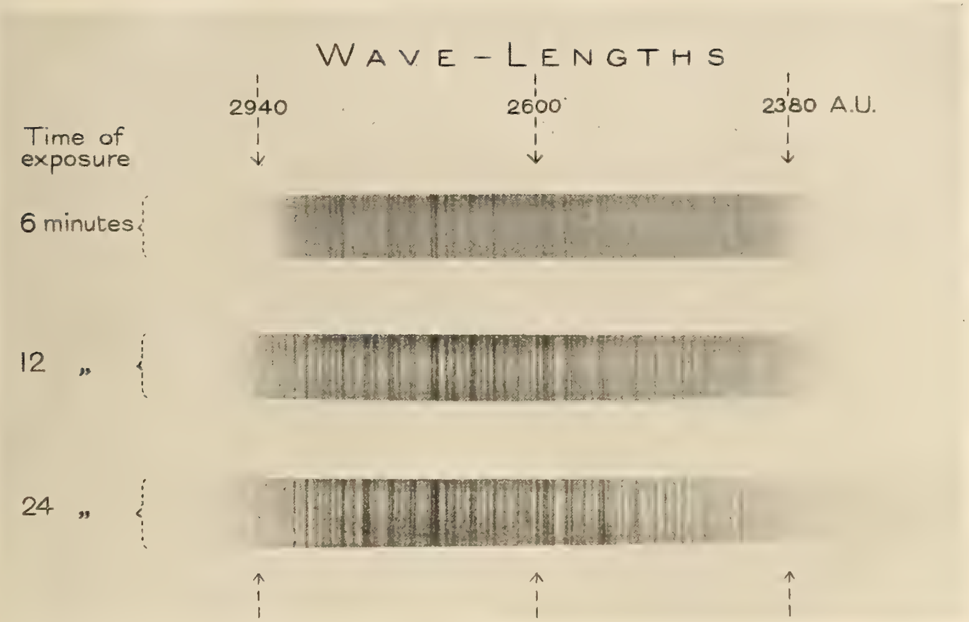
WAVE-LENGTHS
2940
2600
2380 A.U.
Time of exposure
6 minutes
12 ,,
24 ,,

FIG. 1.

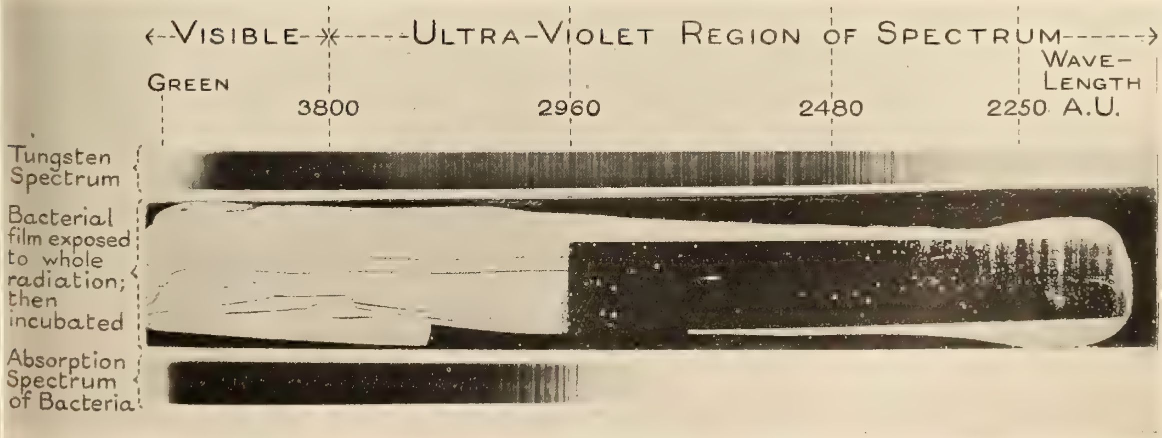
←Visible→←Ultra-Violet Region of Spectrum→
Green
3800
2960
2480
2250 A.U.
Wave-Length
Tungsten Spectrum
Bacterial film exposed to whole radiation; then incubated
Absorption Spectrum of Bacteria

FIG. 2.

The Isolation from Peat of Certain Nucleic Acid Derivatives.

By W. B. Bottomley, M.A., Professor of Botany, University of London, King's College.

(Communicated by F. W. Oliver, F.R.S. Received May 31, 1917.)

In a previous communication* describing the stimulating effect of certain organic substances, extracted from "bacterised" peat, on the growth of *Lemna minor* in water-culture solution, it was suggested that some of the substances may act directly as organic nutrients, being absorbed as such and utilised directly for building up the protein and other complex nitrogenous constituents of the plant.

The marked effect of these substances on the development of the nucleus in the cells of the *Lemna minor* plants also suggested the possible presence of some nuclear constituent, such as nucleic acid, in the extracts. An examination of the aqueous extract of "bacterised" peat showed that, although it contained no nucleic acid, certain purine and pyrimidine bases, together with phosphoric acid, were present. As the presence of these free radicles indicated the possibility that nucleic acid exists as such in raw peat, and is decomposed by further bacterial treatment, an attempt was made to isolate nucleic acid from raw peat.

The usual method of obtaining nucleic acid from soil is by precipitating an alkaline extract of soil with hydrochloric acid, filtering off the precipitate, and pouring the filtrate, after concentration *in vacuo*, into an excess of alcohol containing sodium acetate. By this method, however, there is danger of precipitating some of the nucleic acid with the humic acid.

Schreiner and Lathrop† in their soil investigations used acetic acid instead of hydrochloric acid to acidify the alkaline extract. This precipitates humic acid, but not the nucleic acid.

A combination of these methods was first employed in the extraction of nucleic acid from raw peat. An alkaline extract was obtained in the usual way by treatment with a 1-per-cent. solution of caustic soda. This was just neutralised with hydrochloric acid, then acidified with acetic acid, and the humic acid filtered off. The filtrate was treated by the ordinary method for the separation of nucleic acid, and a flocculent precipitate obtained. This substance was much darker in colour than yeast nucleic acid, and was evidently impure, for on solution and reprecipitation much of the colouring matter was removed.

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* 'Roy. Soc. Proc.,' B, vol. 89, p. 481 (1917).

† Schreiner and Lathrop, 'U.S. Dept. Agric., Bureau of Soils, Bull. 89' (1912).

This method was found to be laborious when working with such a substance as peat, owing to the large amount of humic acid brought into solution by the caustic alkali employed, and the difficulty of rapidly removing this after precipitation.

After numerous experiments it was found that when peat is extracted with a solution of sodium bicarbonate the humic acid remains undissolved, and there is separated from the filtrate a product similar to that obtained by extraction with caustic soda.

Accordingly, a quantity of finely-ground air-dried raw peat was saturated with a 1-per-cent. solution of sodium bicarbonate, and allowed to stand for 24 hours. The liquid was then expressed, and the peat again extracted with a similar solution. The combined extracts were filtered, just neutralised with hydrochloric acid, concentrated *in vacuo* to a small volume, and then poured into about four volumes of absolute alcohol containing sodium acetate and hydrochloric acid. The flocculent precipitate was allowed to settle for 24 hours, and after decanting the supernatant liquid through a filter, the precipitate was washed and dried in a vacuum desiccator. The combined filtrate and washings were preserved for examination.

Examination of the Precipitate.

From the method of preparation, the substance obtained was thought to be nucleic acid, and on incineration of a portion a considerable amount of phosphoric acid was obtained from the ash. Mild hydrolysis with mineral acids, however, failed to yield more than a trace of a purine base, but the hydrolysed product reduced Fehling's solution and gave Molisch's reaction for carbohydrates. The failure to obtain definite purine substances indicated that the product was not similar in nature to yeast nucleic acid, which readily yields purine bases on mild hydrolysis with mineral acids. An examination of the alcoholic filtrate showed that it also contained phosphoric acid, sugar, and a purine base.

As this mild hydrolysis failed to yield an appreciable quantity of purine bases, although the substance was highly nitrogenous, the method of stronger hydrolysis described by Jones* for obtaining pyrimidine derivatives was employed. This method also separates the purine bases, if present, as a silver compound.

(a) *Separation of Purine and Pyrimidine Bases.*—Twenty grammes of the substance were heated with 150 c.c. of 25-per-cent. sulphuric acid in an autoclave for five hours at 140° C. The product was diluted with distilled water to 400 c.c., and hot saturated barium hydroxide added in excess to the boiling solution. This precipitated the sulphuric and phosphoric acids, together with much of the colouring matter. Excess of barium hydroxide was removed by carbon dioxide, and the yellow filtrate concentrated to 150 c.c. This was acidified with nitric acid, and silver nitrate added as long as a precipitate formed. This silver-purine precipitate was removed by filtration, and to the cooled filtrate silver nitrate was further added until a drop of cold saturated barium hydroxide produced a yellow precipitate. Barium hydroxide was then added until the solution was permanently alkaline and precipitation ceased.

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* Jones, W. 'Nucleic Acids,' p. 90 (1914).

(b) *Examination of the Silver-Pyrimidine Precipitate.*—The precipitate was filtered by means of a pump, suspended in hot water and decomposed with hydrogen sulphide. A trace of barium was quantitatively removed with sulphuric acid, and the filtrate from the silver sulphide concentrated to a small volume. A hot saturated solution of picric acid was then added, but after standing for 48 hours no precipitate of cytosine picrate appeared. The picric acid was therefore extracted with sulphuric acid and ether, and, after removal of most of the sulphuric acid by barium hydroxide, the liquid was again concentrated, when fine needle clusters formed. The liquid also gave the pyrimidine colour reaction of Wheeler and Johnson.

The method of preparation of this substance, its failure to form an insoluble picrate, the needle clusters formed in sulphuric acid solution, together with the Wheeler and Johnson colour reaction, identify it as uracil.

(c) *Examination of the Silver-Purine Precipitate.*—The silver-purine compound was suspended in hot water, decomposed with hydrochloric acid, and the precipitate of silver chloride filtered off. A portion of this filtrate was tested for guanine by adding excess of ammonia at the boiling point. As no trace of a precipitate of guanine was obtained, to another portion of the filtrate picric acid was added and a drop or two of ammonia, when a copious precipitate of fine needles at once appeared. This was filtered off and recrystallised from hot water, when the crystals appeared in the form of prisms. These were dried and were found to melt with effervescence and decomposition at 277° C., the melting point of adenine picrate. This substance was further identified as adenine by the characteristic formation of the following salts: the bichromate, as six-sided plates; the double salt with gold chloride, as long orange coloured prisms; the hydrochloride, as flat deliquescent prisms. The substance also gave a gelatinous precipitate with ammoniacal silver nitrate, a fine red colour with ferric chloride, unchanged by heating, and responded to Kossel's test for purine bases.

During the process of hydrolysis a copious precipitate of humic acid was formed, presumably from the carbohydrate radicle. The filtrate, however, still gave a strong reducing reaction with Fehling's solution and responded to Molisch's test for carbohydrates. It also gave positive results with the phloroglucin and orcin tests for pentoses.

The hydrolysed product also gave a strong reaction for phosphoric acid with both magnesia mixture and ammonium molybdate.

The material therefore contains phosphoric acid, a pentose sugar, one purine and one pyrimidine base. This indicates that it is a dinucleotide—an adenine-uracil dinucleotide—and not the typical tetranucleotide, nucleic acid.

Examination of the Filtrate.

(a) *Separation of the Purine Base.*—A preliminary test having shown that the filtrate from the dinucleotide contained phosphoric acid, sugar and a purine base, a further examination of the liquid was made. The alcohol was distilled off and a small portion of the aqueous residue was made alkaline with ammonia. A flocculent precipitate formed which settled as a crystalline sediment. After standing for 24 hours this was filtered off, dissolved in a small amount of boiling hydrochloric acid, its colour discharged with animal charcoal, and the base, together with the phosphoric acid, reprecipitated with ammonia at the boiling point. A small proportion of this precipitate, evaporated on porcelain with a drop of nitric acid, left a muddy yellow spot which gave a brownish-red colour with sodium hydroxide. This indicated the presence of guanine.

The whole of the filtrate was then treated in a similar way and the precipitate dissolved in hydrochloric acid. From this solution of the chloride a mixture of elongated tetrahedral and needle crystals was obtained on long standing in a desiccator. This chloride was used for the preparation of the following salts: the picrate, as a woolly mass of long, fine, thread-like needles, which dried to a felt-like mass and on heating became orange-red, decomposing without melting at 190° C.; the bichromate, as bright orange coloured prisms with truncated ends, which became dark violet when heated at 100° C. These reactions, together with its solubility in ammonia and hydrochloric acid and the formation of a gelatinous precipitate with ammoniacal silver nitrate, identified the substance as guanine.

Guanine was also isolated from the filtrate by precipitation with copper sulphate and sodium bisulphite in the ordinary way. No other purine base could be isolated from the filtrate.

(b) *Separation of the Pyrimidine Base.*—A small portion of the filtrate after

PROCEEDINGS
OF
THE ROYAL SOCIETY.

Series B. Vol. 90. **No. B 624.**

BIOLOGICAL SCIENCES.

CONTENTS.

Page

The Distribution in Wheat, Rice, and Maize Grains of the Substance, the Deficiency of which in a Diet causes Polyneuritis in Birds and Beri-beri in Man. By HARRIETTE CHICK and E. MARGARET HUME . . 44

The Effect of Exposure to Temperatures at or above 100° C. upon the Substance (Vitamine) whose Deficiency in a Diet causes Polyneuritis in Birds and Beri-Beri in Man. By HARRIETTE CHICK and E. MARGARET HUME 60

Two Cases of Congenital Night-Blindness. By Sir W. DE W. ABNEY, K.C.B., F.R.S. 69

Obituary Notices of Fellows deceased :—Daniel Oliver (with portrait) ; Captain Geoffrey Watkins Smith . . . . xi—xix

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PRINTED FOR THE ROYAL SOCIETY AND SOLD BY
HARRISON & SONS, 45, ST. MARTIN'S LANE, LONDON, W.C.

Price Two Shillings and Sixpence.

No. B 624.

December 1, 1917.

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the precipitation of the guanine by ammonia was examined by the method of Jones, already described, for pyrimidines, and cytosine was obtained.

A simpler method for the isolation of this base was found in the use of mercuric sulphate, which dispenses with the need of a silver salt. The bulk of the filtrate was therefore acidified with sulphuric acid and a solution of mercuric sulphate added. After standing for 24 hours the precipitate was removed by filtration, decomposed with hydrogen sulphide, and the filtrate from the mercuric sulphide concentrated. From this the following salts of cytosine were obtained: an almost insoluble picrate in the form of needles; a chloroplatinate, as six-sided plates; a chloride, as needle crystals. The free base was obtained in the form of pearly plates by precipitating with ammonia, and this gave the colour reaction of Wheeler and Johnson. By these reactions the substance was identified as cytosine.

Thus the filtrate contains the four radicles of another dinucleotide—phosphoric acid, sugar, guanine and cytosine—a guanine-cytosine dinucleotide.

Various kinds of peat from different localities and varying depths have been extracted, and all have given similar results.

Conclusions.

It is evident from the above results that all the constituents of a true nucleic acid are present in raw peat, but nucleic acid as such has not been isolated. Nucleic acid must have been present in the plants from which peat has been formed, and since it is improbable that hydrolysis could have been brought about by the methods of extraction employed, the original nucleic acid has evidently been decomposed by bacterial or other agencies during the process of peat formation, into the products which have been isolated.

It is generally assumed that the first step in the decomposition of nucleic acid results in the formation of four mononucleotides. Levene and Medigreceanu* state that the first phase in the enzymatic decomposition of yeast nucleic acid is the formation of four mononucleotides by the action of a ferment called "nucleinase." It is evident, however, that the decomposition of peat nucleic acid *in situ* has not followed these lines, but has yielded a stable adenine-uracil dinucleotide and the component parts of a guanine-cytosine dinucleotide.

This is interesting in view of the recent work of Jones and Germann,† who have shown that when yeast nucleic acid is submitted to alkaline hydrolysis it is split up into two very definite dinucleotides: a guanine-cytosine dinucleotide and an adenine-uracil dinucleotide. They state that the former easily hydrolyses into its component mononucleotides, but the latter is comparatively stable. Evidently a similar decomposition of nucleic acid has taken place during peat formation, and the relatively unstable guanine-cytosine dinucleotide has been further decomposed into its constituent parts.

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* Levene and Medigreceanu, 'Journ. Biol. Chem.,' vol. 9, p. 65 (1911).

† Jones and Germann, 'Journ. Biol. Chem.,' vol. 25, pp. 98 *et seq.* (1916).

In view of the stable nature of this adenine-uracil dinucleotide and the ease with which it can be extracted from peat, it is surprising that its occurrence as such in nature has hitherto escaped observation.

So far the work has been purely qualitative, but a more exhaustive examination of the substances obtained is now in progress, to determine not only the quantitative proportions of the various radicles, but also their elementary composition.

The Distribution in Wheat, Rice, and Maize Grains of the Substance, the Deficiency of which in a Diet causes Polyneuritis in Birds and Beri-beri in Man.

By Harriette Chick and E. Margaret Hume.

(Communicated by Dr. C. J. Martin, F.R.S. Received January 10, 1917.)

(From the Lister Institute.)

Our attention was turned to this subject by the reported occurrence of beri-beri among our forces in the Dardanelles and Mesopotamia during the autumn and winter of 1915 (see Wilcox, 1916, I and II). Owing to the exigencies of the military situation, many individuals in these regions subsisted for considerable periods mainly on tinned meat, jam and white bread (or biscuit baked principally from white flour). Beri-beri has in recent years been included among the deficiency diseases, and there was therefore good presumptive evidence that the diet had been at fault. We examined two samples of tinned meat-and-vegetable ration by the methods described below, and found, as was to be expected, that the substances preventing beri-beri in the fresh materials had not survived the heat-sterilisation undergone in the process of canning. The case of bread and biscuit demanded a more extended investigation, and the present work is concerned with the nutritive properties of cereals generally, especially wheat.

One member of this group, viz., rice, has been exhaustively studied in recent years, and the work, among others, of Eijkman, Grijns, and Braddon has established the etiology of tropical beri-beri in a deficiency in the diet of rice-eating people, caused by the removal of certain materials from the grain during milling. The presence in the rice-bran or rice polishings of a substance essential for nutrition has been demonstrated by many observers, including those named above, and also by Schaumann (1910), to whom and to Funk (1913) the reader is referred for a complete bibliography upon the subject. Funk (1912) adopted the term "vitamine" for this essential material, and for the sake of brevity we shall use the expression "anti-neuritic vitamine"* to express the substance whose absence in a diet causes beri-beri. The vitamine in the rice grain was supposed to be contained in the layer of cells rich in protein (aleurone-layer) situated immediately underneath the pericarp of the husked grain. These cells form the outer layer of the endosperm, but are removed with the bran during milling.

As a result of experiments made chiefly with wheat, but also including rice and maize for purposes of comparison, we have reached the conclusion that the more important receptacle of the anti-beri-beri vitamine is the embryo or germ, and not the pericarp, of the grain. In the case of wheat, there are already some facts in the literature which indicate such a conclusion, but the exact composition of the materials employed in those researches was not sufficiently controlled for any dogmatic conclusion to be drawn. For example, Holst (1907) and Edie and Simpson (1911) have shown that a diet of white bread (milled wheat-endosperm) will induce polyneuritis (beri-beri) in pigeons in the same manner as polished rice (milled rice-endosperm), and the latter observers proved that birds would remain well and healthy if (*a*) whole-meal bread or (*b*) "standard" bread is used instead. The last-named article, however, in spite of its title, has no standard composition; it is generally made from white flour containing an addition of wheat germ and comparatively little bran. Hill and Flack (1911) demonstrated the inferior nutritive properties of white bread compared with "whole-meal" or "standard" bread in the case of young rats. They further showed that satisfactory growth could be induced if a proportion of wheat germ were added to a diet of white bread, and it was at Dr. Flack's suggestion that we turned our attention to wheat germ in the hope that the subject would repay study. Quite recently McCollum and Davis (1915) have found that young rats will thrive upon a diet of polished rice, butter fat and salts to which either wheat embryo or rice polishings are added; they have suggested that the curative value of rice polishings for polyneuritis of birds might be attributed to the rice embryo removed with the pericarp rather than to the bran itself.

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* This expression is also convenient to mark a distinction from those substances equally essential in a human diet, for the prevention of scurvy, which may be referred to as "anti-scorbutic vitamines."

The modern "roller" milling of wheat effects so perfect a separation of the constituent parts of the grain that trustworthy material for experiment is readily obtained. During the "break" of the grain by the rollers the pericarp, being brittle, is broken up into bran, while the embryo, being soft and oily, is squeezed out flat. The subsequent operations of milling, which include such processes as sieving, fanning and centrifuging, lead to a complete separation of these two products. With rice, however, this is not the case, and the few experiments we made with rice germ were made with materials separated by hand in the laboratory.

Methods Employed.

Polyneuritis of birds has been accepted as analogous to beri-beri in human beings with regard to etiology, symptoms, and methods of cure. The following experiments were, therefore, made with pigeons of 300–400 grm. weight, and were of two kinds, the methods adopted being in principle those used by Cooper (1913 and 1914), working in this Institute.

(1) *Preventive,* in which determination was made of the minimum amounts of the various materials in question, that must be added to a (relatively) vitamine-free diet in order to prevent the occurrence of polyneuritis. Polished rice, about 40 grm. daily, formed the vitamine-free diet, and prevention was considered to be successfully accomplished if the bird showed no symptoms of polyneuritis after a period of about 60 days. Unprotected birds usually developed symptoms in 15–25 days.

(2) *Curative,* in which determination was made of the minimal amount which, administered by the mouth, would cure a bird suffering from acute polyneuritis. A bird in this condition, brought on by an exclusive diet of polished rice, will usually die within 24–48 hours if not treated. In order to be able to administer the requisite amount of curative substance in time Cooper (*loc. cit.*) concentrated the vitamines by extracting the air-dried material with absolute alcohol in the cold and evaporating the alcoholic extract to dryness under reduced pressure. This residue was taken up in a small volume of water and definite quantities were given by the mouth, the doses being reckoned in terms of the original foodstuffs. We found it possible, however, in most cases, to give the curative doses of the original materials without preliminary extraction. By this means we gained a better idea of their absolute vitamine value in the natural condition, and avoided the loss due to the extraction processes. Our method, however, has its drawbacks, as, unless the crop of the bird is fairly empty at the time when a cure is essayed, digestion and absorption will be slow and difficult. In addition, these processes are frequently interfered with by the paralysis of the bird, so that cures are often slow (24 hours to 3 or 4 days) in progress, and it is unusual

to see the swift dramatic changes (in 6–24 hours) so typical of the cure of polyneuritis in birds, when the vitamine is administered in a soluble form. Nevertheless, with the exception of a small proportion of cases, where the character of the symptoms and the general condition were unsuitable, cures can usually be accomplished if the amount of vitamine given is adequate. Some examples are given in Tables V and VI below, in which similar material was used for cures both before and after concentration of the active substance by extraction. In the case of wheat germ, the curative dose was found to be equal to 2·5 grm. of the original material; after extraction, the equivalent of 15 grm. was required, which result shows the large proportion of vitamine lost by the extraction process.

In the case of comparative curative experiments the difficulty is, firstly, to form a judgment of the severity of the symptoms, and, secondly, to obtain a sufficiency of acute cases in which this shall be approximately equal. This difficulty was not altogether overcome, as will be seen in the somewhat irregular results of the experiments described below, especially those set forth in Table VI. Preventive experiments form the more satisfactory method of estimating the vitamine-content in foodstuffs and yield much more regular results. The drawback is that they take at least two months to complete, and, if adopted exclusively, would limit the scope of any enquiry.

Feeding was artificial in both cases and the rice rations given in the Tables must be regarded as approximate only, since the birds frequently vomited small amounts after feeding. In case of the wheat-bran and wheat-germ rations, no significant amount of vomiting was noticed, nor was the rice vomited when these substances were added to the diet.

Wheat Embryo.—Tables I and II give the results of preventive experiments with two different samples of wheat germ, and in each case a series of birds was arranged to act as control, with a diet consisting exclusively of polished rice. The results of these two sets of experiments are in concordance; in both a ration of 3 grm. wheat germ, given every second day, was found to afford complete protection from polyneuritis. With 2 grm. every second day, half the birds used in each experiment were protected.

Wheat Bran.—The wheat bran used for comparison with the wheat germ was what is known as "Broad bran," and the sample R.I. (Table III) was obtained from the same wheat as germ R.I. (Table II) and white flour R.I. (Table IV). It consisted of large particles, and in its thickness comprised all the outer layers of the grain. Traces of the starchy endosperm were visible upon the inside surface, and the presence of the aleurone-layer was demonstrated by sections. The sample R.I. was obtained from a modern

Table I.—Minimum Ration of Wheat Germ which must be added every Second Day to a Diet of Polished Rice in order to prevent Onset of Polyneuritis in Pigeons (300–400 grm.). Sample A; Water Content 13 per cent.

Experiment No.	Germ ration every second day.	Rice ration daily.	No. of bird.	Time elapsing before onset of polyneuritis.		Weight changes.				Remarks.	
						Original weight of bird.	Final weight of bird.	Change in weight during period of experiment.			
	grm.	grm.		days.	mean, days.	grm.	grm.	per cent.	mean, per cent.		
Control	0	About 40	81	18		329	284	−14			
			82	15		295	290	− 2			
			83	12		285	237	−17			
			84	15		355	337	− 5			
			85	26		280	234	−16			
			86	13		279	259	− 7			
			87	23		384	289	−25			
			88	13	17	319	284	−11	−12		
1	0·5	30 40	70	26		400	284	−29		No protection.	
			71	25	25·5	385	324	−16	−22	No protection.	
2	1·0	30–40	72	17		354	279	−21		No protection.	
			76	28	22	393	342	−13	−17	No protection.	
3	2·0	30-40	74	More than 67		352	272	−23		Two birds protected out of four	—
			75	57–67		345	277	−20			Slight chronic symptoms after 57th day.
			95	31		385	330	−14			—
			96	More than 63		312	290	− 7	−16		Remained without symptoms for 8 days after discontinuing germ ration.
4	3·0	30–40	92	More than 63		307	280	− 9		All birds protected	Remained without symptoms for 11 days after discontinuing germ ration.
			93	More than 63		312	312	0			Remained without symptoms for 16 days after discontinuing germ ration.
			94	More than 63		310	304	− 2	− 4		Remained without symptoms for 18 days after discontinuing germ ration.
5	4·0*	30–35	77	More than 63		370	429	+16			Remained without symptoms for 8 days after discontinuing germ ration.
			78	More than 63		292	310	+ 6	+11		Remained without symptoms for 10 days after discontinuing germ ration.
6	6·0*	30–35	79	More than 63		367	460	+25			Remained without symptoms for 15 days after discontinuing germ ration.
			80	More than 63		385	522	+36	+30		Remained without symptoms for 12 days after discontinuing germ ration.

* These rations were given as 2 grm. and 3 grm. daily, respectively, for purposes of convenience; 6·0 grm. germ is an excessive meal for a bird of this size.

Table II.—Minimum Ration of Wheat Germ which must be added every Second Day to a Diet of Polished Rice in order to prevent Onset of Polyneuritis in Pigeons (300–400 grm.). Sample R.I.; Water Content 16 per cent.

Experiment No.	Germ ration every second day.	Rice ration daily.	No. of bird.	Time elapsing before onset of polyneuritis.		Weight of bird.		Change in weight during experiment.		Remarks.	
						Original.	Final.				
	grm.	grm.		days.	mean, days.	grm.	grm.	per cent.	mean, per cent.		
Control I	0	About 40	160	16		305	285	− 7			
			161	14		380	332	−13			
			162	17		342	319	− 7			
			163	21		322	277	−14			
			164	26		362	288	−20			
			165	13	18	315	306	− 3	−11		
1	1·5	30–40	166	40		369	290	−21		Some degree of protection, mean time of onset of polyneuritis delayed about two weeks.	
			167	32		340	299	−12			
			168	49		360	244	−32			
			169	20	35	397	321	−19	−21		
2	2·0	30–40	170	More than 63		312	250	−20		Two birds protected out of four	Protection not complete, slight chronic lameness during latter part of experiment.
			171	44		362	354	− 2			—
			172	More than 63		390	360	− 8			Remained without symptoms for 5 days after discontinuing germ ration.
			173	26		395	355	−10	−10		—
Control II	0	About 40	200	17		387	298	−23			
			201	17		332	312	− 6			
			202	22		374	282	−25			
			203	22	19·5	385	297	−23	−19		
3	3·0	30–40	196	More than 63		320	330	+ 3		Complete protection	Remained without symptoms for 7 days after discontinuing germ ration.
			197	More than 63		345	382	+11			Remained without symptoms for 8 days after discontinuing germ ration.
			198	More than 63		397	380	− 4			Remained without symptoms for 7 days after discontinuing germ ration.
			198	More than 63		347	422	+22	+ 8		Remained without symptoms for 8 days after discontinuing germ ration.

Table III.—Influence of Regular Ration of Wheat Bran ("Broad Bran") upon Onset of Polyneuritis in Pigeons (300–400 grm. weight) fed upon an Exclusive Diet of Polished Rice.

Description of sample.	Bran ration every second day.	Rice ration daily.	No. of bird.	Time elapsing before onset of polyneuritis.		Weight of bird.		Change in weight during period of experiment.		Remarks.
						Original.	Final.			
	grm.	grm.		days.	mean, days.	grm.	grm.	per cent.	mean, per cent.	
"R.I." (corresponds to germ "R.I." and white flour "R.I.") free from germ, roller-milled, water content 12 per cent.	0	About 40	212	16		490	365	−26		
			213	16		380	317	−17		
			214	20		432	340	−21		
			215	19	18	460	350	−24	−22	
	3 ·0	30–40	216	20		465	410	−12		No protection, mean time of sickening approximately equal to control.
			217	18		348	372	+ 7		
			218	26		490	360	−27		
			219	14	19 ·5	336	288	−14	−11 ·5	
	0	About 40	244	18		465	396	−15		
			245	21		493	425	−14		
			246	21		400	295	−26		
			247	18	19 ·5	410	316	−23	−19 ·5	
	5 ·0	30–40	248	51		375	285	−24		Small degree of protection, mean time of onset of polyneuritis delayed about two weeks.
			249	23		415	330	−20		
			250	27		398	385	− 3		
			251	40	35 ·5	429	352	−18	−16	
Sample A, stone-milled, probably not free from germ	0	About 40	178	14		260	225	−13		
			179	26	20	325	272	−16	−14 ·5	
	5 ·0*	30–40	174	47		324	322	− 1		Some degree of protection, time of onset of polyneuritis delayed about four weeks.
			175	48	48	284	265	− 7	− 4	
	10 ·0*	30–40	177	More than 63		246	230	− 6		Protection.

* Ration given as 2 ·5 grm. and 5 grm. daily, respectively, owing to the bulky nature of the material.

roller-mill, and may be regarded as free from germ. Birds receiving a ration of 3 grm. every second day developed polyneuritis in the same time as those upon an exclusive polished rice diet; with 5 grm. every second day the onset of symptoms was postponed for about two weeks. If the results of Table III be compared with Table II one may regard the anti-neuritic value of 5 grm. of broad bran as equivalent to 1·5 grm. germ.

On general grounds it would seem not unlikely that what value the bran possesses must be attributed to the aleurone-layer, which forms only a small proportion of the whole. Hamill (1911) states that the aleurone-layer forms about 4 per cent. of the grain and the pericarp about 15 per cent. He gives no authority for these figures, and it is difficult to see that they can be other than approximate. On this basis, the aleurone-layer would constitute about one-fifth of the bran, and, weight for weight, be about equal to the embryo as regards vitamine-content.

It is interesting to note the greater anti-neuritic power of the broad bran derived from stone-milled wheat (see Sample A, Table III). In this case no separation of the germ is effected in the milling, and each constituent issuing from the mill contains some of it.

Table IV.—Influence of a Diet of "Straight Run" White Wheat Flour in Comparison with a Diet of Polished Rice. Sample of Flour "R.I.," roller milled, free from germ and bran, from same wheat as Germ R.I. and Broad Bran R.I.

White flour ration, daily.	Polished rice ration, daily.	No. of bird.	Time elapsing before onset of polyneuritis.		Weight of bird.		Change in weight during period of experiment.	
					Original.	Final.		
grm.	grm.		days.	mean, days.	grm.	grm.	per cent.	mean, per cent.
0	About 40	256	15		416	387	− 7	
		257	32		450	345	−23	
		258	18		455	370	−19	
		259	14		449	372	−17	
		262	30	22	507	365	−28	−19
20–30	0	260	16		506	465	− 8	
		261	28		515	402	−22	
		263	13	19	485	435	−10	−13

Wheat Endosperm.—In Table IV are given the results on pigeons of an exclusive diet of "straight run" white flour, or starchy wheat endosperm (after removal of the aleurone-layer), the sample employed, viz., R.I., corresponding to bran R.I. (Table III) and germ R.I. (Table II). The birds received 20–30 grm. daily, made into pills after mixing with a minimal amount of water. By experience this ration was found to be as much as they could assimilate. Polyneuritis occurred in each instance, and the time of onset was, if anything, rather earlier than that occurring with a series of control birds fed on polished rice. There was also no significant difference in the loss of weight suffered by the birds in the two series of experiments.

Curative Experiments.

In Table V are given the results of some curative experiments made with (1) wheat germ, and (2) wheat "middlings," or the finely ground bran, which is probably not free from admixture with germ. In both cases the vitamines were concentrated by the extraction process with alcohol described above (p. 46). The relative curative values of these two materials were found to be in the ratio of about 5·5 to 1, if we judge by the "great improvement" obtained with the extracts equivalent to 9 and 50 grm. respectively in the two instances.

It is necessary to define here what is meant by the various types of cure described in Tables V and VI. The expression "complete cure" indicates that both flight and gait were perfectly restored; "cure" that one or both remained slightly imperfect, while "incomplete cure" is used to describe a rather less satisfactory condition. The birds before treatment were acutely

Table V.—Comparison of Minimum Amounts of Wheat Germ and Wheat Bran required to cure Pigeons (300–400 grm.) suffering from Acute Polyneuritis induced by a Diet of Polished Rice. The materials were extracted with alcohol and the residues obtained, after evaporation of the alcohol, taken up with water. Doses reckoned in amounts of the original foodstuff taken.

Description of material.	No. of bird.	Amount of curative dose given.		Result.
		In terms of natural foodstuff.	In terms of dry weight.	
		grm.	grm.	
Wheat germ, Sample A, water content 13 per cent.	88	10	8·7	Great improvement.
	83	15	13·0	Complete cure.
	56	15	13·0	Complete cure.
	59	20	17·4	Complete cure.
"Middlings," or fine wheat bran, probably not free from germ, water content 11 per cent.	104	28	25	Improvement, no cure.
	114	56	50	Improvement, no cure.
	129	56	50	Great improvement.

Table VI.—Minimum Amount of Wheat Embryo required to cure Pigeons (300—400 grm. weight), suffering from Acute Polyneuritis induced by a diet of Polished Rice. Material administered by the mouth, without preliminary extraction, as boluses made with a little water.

Description of material.	No. of bird.	Amount of curative dose.		Result.	No. of days cure lasted.	Nature of symptoms.	Time of sickening.
		In terms of foodstuff.	In terms of dry weight.*				
		grm.	grm.				days.
Wheat-embryo, Sample B, containing about 15 per cent. fine bran; water content 11 per cent.	147	1·0	0·9	Improvement	3	Lameness	23
	161	1·0	0·9	Complete cure	5	Head symptoms	14
	162	1·0	0·9	No improvement, died	—	Head symptoms and crop-bound	17
	220	1·0	0·9	Died after 24 hours	—	Head symptoms and lameness	23
	221	1·0	0·9	Died after 48 hours	—	Head symptoms, lameness and crop-bound	23
	131	2·5	2·2	Complete cure	9	Head symptoms and lameness	24
	164	2·5	2·2	Very slight improvement for 24 hours	—	Lameness and crop-bound	26
	232	2·5	2·2	Died	—	Head symptoms	12
	234	2·5	2·2	Complete cure	9	Head symptoms	18
	236	2·5	2·2	Complete cure	8	Head symptoms	22

* After heating to 100–110° C.

ill, and were usually quite helpless; by the term "improvement" is meant that the acute symptoms subsided, and that the bird was out of danger for a time. It should be added, perhaps, that these expressions deal with symptoms alone, and that nothing is assumed with regard to the actual lesions. It is obviously impossible that if axon degeneration is present this can be restored in the short period occupied by the "cure." The quality of the cure was also studied by continuing the diet of polished rice and noting the time elapsing before the recurrence of polyneuritis symptoms.

Further curative experiments with wheat germ are set forth in Table VI. Here no extraction with alcohol took place, but the moistened germ was given in the form of boluses, which were readily swallowed by the bird. The results of this experiment, compared with those given in Table V, show how great is the loss of vitamine due to the extraction process, and how small, comparatively (from 1 to 2·5 grm.), is the amount of wheat germ required to effect a cure when given in the natural condition.

The results also show the degree of irregularity that must be expected in this type of experiment (see above, p. 47). This is doubtless due to differences in the nature and severity of the symptoms in the different birds, all of whom were acutely ill, and in certain cases also to difficulty in digesting and absorbing the curative material. Those cases in which the action of the crop was affected by polyneuritis are by far the hardest to cure, and probably should be disregarded in any attempt to estimate vitamine-content in the substances administered as cures.

From Table VI one may conclude that 2·5 grm. of wheat embryo may generally be relied upon to cure acute polyneuritis in a pigeon of 300–400 grm. weight; at the same time, cures were obtained, exceptionally, with 1 grm., and occasionally with 1·5 and 2 grm.

For comparison with the above, some curative experiments were made with "broad bran," this being again chosen as likely to provide a specimen of pericarp free from any admixture of germ. In one case we failed to effect a complete cure with 10 grm., and in another we obtained no improvement with 5 grm., while in a third instance we obtained a cure with 5 grm. These experiments present special difficulties as regards their correct interpretation; the bran is very bulky, the dose is large, and difficulties of absorption correspondingly enhanced. In fact, a much more trustworthy estimate of the relative vitamine values of wheat pericarp and embryo, weight for weight, are obtained when the vitamines are concentrated and administered in a soluble form as in Table V (1 : 5·5), or, better still, when the results of preventive experiments are used to make the comparison (1·5 : 5, see Tables I–III).

Rice.

We have only made a few preliminary experiments with rice, owing to the difficulty in obtaining material. Some little work has, however, been accomplished, using rice embryo separated from the unmilled grain by hand, in the laboratory. The rice embryo is extremely small, and amounts only to about 2·5 per cent. by weight of the husked grain, so that the work is exceedingly laborious; only a small amount of material was obtained, and all the experiments were perforce of the curative type. The rice-bran used for comparison was a sample kindly furnished by a firm of rice millers, and contained both pericarp and embryo. The experiments with embryo demonstrated the high concentration of vitamine in this organ; the birds showed some improvement in symptoms after administration of 0·3 grm., and better results, almost amounting to cures, after 0·5 grm., while a dose of 1 grm. gave a complete cure lasting nine days. In case of the bran, a distinct improvement was manifested on one occasion after receiving 4 and 6 grm. respectively. In one case improvement was also noticed after 5 grm., and in a second case, not so severe as the first, a complete cure was effected. Complete cures were obtained after doses of 8 and 10 grm. on more than one occasion.

Maize.

A few trials have been made with maize embryo, which also has proved to be very rich in anti-neuritic vitamine. In this cereal the embryo is comparatively large, and it was easily picked out by hand, especially if the grain were soaked in water overnight. After this treatment the embryo amounted to 15 per cent. by weight of the entire grain. In the case of maize, the plumule (shoot) and radicle (root) of the embryo can be separated with ease from the scutellum, which organ is exceedingly large, and amounts to about 12·5 per cent. of the soaked grain, while the plantlet comprises the remaining 2·5 per cent. Both parts of the embryo, however, proved to be receptacles of vitamine. Cures were achieved with 1 and 2 grm. of the wetted plantlet (equivalent to 0·6 and 0·7 grm. dry weight respectively). A complete cure, lasting 13 days, followed a dose of 2 grm. (dry weight) of the entire germ (scutellum, plumule, and radicle), and a less satisfactory result with 3 grm. (dry weight) of scutellum. Smaller quantities than these were not given, and it is hoped later to investigate this cereal further.

Relation between the Preventive Ration and the Curative Dose.

A very curious result has emerged from the foregoing experiments, viz., the very close approximation between the daily ration of wheat germ required to prevent the onset of polyneuritis, and the dose necessary to cure a bird in the acute condition. While 3 grm. must be added every second day to a diet of polished rice, to prevent polyneuritis with certainty (see Tables I and II), the curative dose is found to be only 2·5 grm., and in exceptional cases 1 grm. sufficed. These curative doses cause disappearance of polyneuritic symptoms, brought on by the exclusive diet of polished rice, and the bird is usually maintained in good health for several days, notwithstanding the deficiency in its food (see Table VI). After this period recurrence of symptoms again takes place.

This relation between the preventive ration and the curative dose is not confined to any particular anti-neuritic substance, but appears to be general. In case of a yeast-extract (water-content = 30 per cent.), the daily preventive dose was found to be from 1 to 2 grm., and the curative dose from 2 to 3 grm., occasionally 1 grm. This result is in accord with the observations of Cooper, who (1913) found that 2·5 grm. pressed yeast (dry weight = 0·5 grm.), given daily, would prevent polyneuritis in pigeons fed otherwise with polished rice. In order to cure pigeons suffering from acute polyneuritis (1914, II), he used autolysed yeast, and found it necessary to administer the equivalent of 3-6 grm. of the original pressed yeast; on a second occasion the equivalent of 1–2 grm. was sufficient. There are no other good instances available, because curative experiments have almost universally been made with material which has been extracted with alcohol to concentrate the vitamine, while, for the preventive experiments, the natural foodstuffs have been used. As pointed out above, there is a great loss of anti-neuritic material in the extraction process as usually carried out. Hence the stated amounts required for the cure of animals show an exaggerated size when reckoned on the original foodstuffs.

That the preventive and curative doses are of the same order of magnitude is perplexing, and demands further investigation. In order to obtain some light upon this point we are making a series of preventive experiments in which the total amount of vitamine is kept constant, but the dosage, *i.e.*, size and periodicity of the dose, is varied. These experiments are not yet completed, but the only interpretation which the facts seem capable of carrying at present is that:

(1) There is an urgent daily need for a small quantity of this vital substance to maintain the metabolism of the nerve tissue;

(2) In the normal condition a considerable store is available somewhere in the animal body upon which to draw; and

(3) This store becomes suddenly exhausted, but can be temporarily restored by the administration of a small quantity from outside.

Influence of a Small Regular Ration of Wheat-Embryo in Maintaining the Weight of Birds Fed on Polished Rice.

It has been shown by numerous observers that birds fed exclusively on polished rice lose consistently in weight. In our experience, the average loss of weight under these conditions varied from 11 to 22 per cent. over a mean period varying from 17 to 22 days (see control experiments, Tables I–IV). When a ration of wheat was given insufficient to prevent the onset of polyneuritis (*i.e.*, from 0·5 to 1·5 grm. every second day), this loss in weight was not exceeded, although the mean period of the experiment lasted from 22 to 35 days (see Tables I and II).

When the wheat ration was adequate for protection against polyneuritis (*i.e.*, 3 grm. every second day), in one instance (Expt. 4, Table I) the average loss of weight was negligible, viz., −4 per cent., and in a second (Expt. 3, Table II) a gain in weight was observed, viz., +8 per cent. The total weight gained by the seven birds in these two experiments was 72 grm., or a gain of about 10 grm. per bird.

In Expt. 3, Table I, and Expt. 2, Table II, where 2 grm. wheat germ was given every second day, and polyneuritis was not prevented with certainty, there was an average loss of weight of 16 and 10 per cent. respectively, or a total loss of 365 grm. among the eight birds studied, *i.e.*, 46 grm. per bird.

The average difference in weight between individuals in the above two series of experiments is therefore 56 grm. This must be referred to a difference in diet amounting to 0·5 grm. wheat germ daily over a period of about 60 days, *i.e.*, a total of 30 grm. per head. It is therefore evident that the addition to a diet of polished rice of wheat germ in amount to prevent polyneuritis has also some influence in promoting the general metabolism of the animal.

This result is in accord with the observation of Schaumann (1911), who found that if a ration of a vitamine-containing foodstuff (such as rice-bran or yeast) were adequate to prevent onset of polyneuritis in birds, when added to a diet of polished rice, loss of weight was prevented, whereas extracts of these foodstuffs prepared by extraction with acid or alcohol, sufficient to prevent polyneuritis, did not maintain weight.

Our experiments with wheat-bran offer another example of this principle. Where the bran was free from germ, as in sample R.I. (Table III), a ration of 5 grm. each second day did not prevent polyneuritis, nor did it maintain weight. In case of sample A, which was stone-milled, and presumably contained traces of germ, a ration equal to 5 grm. each second day more nearly protected a pigeon from polyneuritis, and there was no appreciable loss of weight.

Cooper (1913) came to the conclusion that the substances preventing polyneuritis and maintaining body weight were separate and might be separately distributed in natural foodstuffs. For certain of these, *e.g.* yeast, ox-heart and ox-brain, the two substances were evenly balanced and the daily ration required to prevent polyneuritis also maintained the weight of the bird. In others, *e.g.* egg-yolk, barley, lentils, this daily ration had to be increased if loss of weight were also to be prevented. In this connection our experiments in which casein was added to a diet of polished rice (set out in Table VII) are of some interest. The casein, prepared from milk by precipitation and subjected to some purification, proved to be vitamine-free, as was expected; the addition of the extra protein had no influence upon the onset of polyneuritis. With the addition to the diet of 3 grm. of this casein daily, however, the weight of the birds was certainly maintained better than was the case with the control birds fed on polished rice alone.

Table VII.—Influence of Addition of Extra Protein (Casein) to a Diet of Polished Rice, upon the Onset of Polyneuritis in Pigeons (300—400 grm. weight).

Casein ration daily.	Rice* ration daily.	Total protein given daily.	No. of bird.	Time elapsing before onset of polyneuritis.		Weight of bird.		Change in weight during period of experiment.	
						Original.	Final.		
grm.	grm.	grm.		days.	mean, days.			per cent.	mean, per cent.
0	About 35	2·4	107	19		369	292	−21	
			108	21		469	339	−28	
			109	22	21	427	395	− 8	−19
0	About 40	2·8	104	23		300	267	−11	
			105	19		359	287	−20	
			108	16	19	339	295	−13	−14
1·5	35	3·9	110	27		365	305	−17	
			111	20		365	289	−21	
			112	21		382	335	−12	
			113	18	21·5	352	300	−15	−16
3·0	35	5·4	114	25		395	340	−14	
			115	18		365	362	− 1	
			116	14		392	385	− 2	
			117	22	20	394	345	−12	− 7

* Protein content = 6·9 per cent. ('Bulletin No. 45, U.S. Department of Agriculture'; Tibble's "Foods," 1912, p. 474).

A ration of wheat germ equal to 4 grm. every second day, which is slightly in excess of that necessary to prevent polyneuritis, had a marked beneficial influence upon the general health and well-being of the birds (Expt. 5, Table I). When this allowance was further exceeded, as in Expt. 6, Table I, where 3 grm. wheat germ were given daily, the effect was even more noticeable. The increase in weight of the two birds employed amounted, in a period of two months, to 25 and 35 per cent. respectively of their original weight, and the birds displayed unusual energy and vitality. They became wilder and more quarrelsome; they could not be kept together in one cage, as was our custom, seeing that they fought one another with great vigour, if permitted. They would also resist being taken up and examined, and if the cage were opened, would usually take up a sideways position at bay, striking out with the wing against any invader. All this demeanour is in very marked contrast to the gentleness characteristic of birds which are artificially fed and subjected to the large amount of handling which is involved in this type of experiment.

Summary and Conclusions.

1. The foregoing experiments deal with the distribution of "anti-neuritic (anti-beri-beri) vitamines" in the various contituents of the wheat, maize, and rice grains. By "anti-neuritic vitamine" is meant the substance whose deficiency in a diet causes polyneuritis in pigeons and beri-beri in man.

2. Wheat-endosperm, after removal of the aleurone-layer in the ordinary milling processes, constitutes white flour. It is deficient in this vitamine, and if used as an exclusive diet will induce polyneuritis in pigeons (or beri-beri in man) in a manner identical with polished rice.

3. In both the rice and wheat grain, the anti-neuritic vitamine is concentrated mainly in the germ or embryo; it is also present to a less degree in the bran (pericarp and aleurone-layer), probably in the aleurone-layer.

4. In case of maize grain, the embryo also possesses marked anti-neuritic properties. Here the scutellum can be separated from the "plantlet" and separately investigated. Both these constituents of the embryo were found to contain anti-neuritic vitamine.

5. The practical results given under 2 and 3 show the importance of including germ in the flour from which wheaten bread or biscuit is made, especially when the diet may consist largely of preserved foods, *e.g.* tinned meats and vegetables, which are deficient in anti-beri-beri vitamines.

6. The daily ration of wheat-germ that must be added to a diet of polished rice in order to prevent the onset of polyneuritis is of the same order of magnitude as the amount which, administered by the mouth, will cure a pigeon acutely ill with polyneuritis, brought on by an exclusive diet of polished rice. This relation is not peculiar to wheat-germ but applies to other foodstuffs containing anti-neuritic vitamines, *e.g.* yeast.

7. The addition of wheat-germ to a diet of polished rice in quantity (3 grm. every second day) sufficient to prevent polyneuritis, also maintained the weight and general health of the bird. Rations in excess of this (2 grm. every day to 3 grm. every day) led to great increase in body-weight and in general well being and vitality of the birds, which, after a short period of this diet, became in remarkably fine condition.

In conclusion, our best thanks are due to Messrs. Steele and Co. (rice millers), to The Hovis Milling Co., and to Messrs. J. and H. Robinson (wheat millers), for kindness in supplying us with material for experiment, and especially to Mr. E. G. Ellis, of the last-named firm, for much valuable advice and assistance. We are also much indebted to Professor A. Harden, F.R.S., for kindly preparing one of the extracts mentioned in Table V, to Captain A. H. Osman, of the Home Forces Pigeon Service, for a generous supply of pigeons for this work, and lastly to our assistant, Mr. A. H. Robins, for his devoted work in connection with the artificial feeding and care of the experimental birds.

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The Effect of Exposure to Temperatures at or above 100° C. upon the Substance (Vitamine) whose Deficiency in a Diet causes Polyneuritis in Birds and Beri-Beri in Man.

By Harriette Chick and E. Margaret Hume.

(Communicated by Dr. C. J. Martin, F.R.S. Received January 10, 1917.)

(From the Lister Institute.)

The influence of exposure to high temperatures upon the "vitamine," whose deficiency in a diet causes polyneuritis in birds and beri-beri in man, is a subject of great practical importance, chiefly for two reasons. In the first place, it is very necessary to know what degree of destruction, if any, is suffered by the vitamines contained in ordinary foodstuffs during the usual cooking processes, at or about 100° C. In the second place, there is the case of preserved foods, such as tinned meat, vegetables, milk, etc., which are sterilised at temperatures above 100° C. in the process of their manufacture.

The fate of the vitamines during this treatment becomes a vital matter where preserved foods form the staple article of a diet, as may be the case with troops on active service, separated from fresh food supplies.

In such circumstances the ration is usually completed with bread or biscuit, and we have made a special investigation of the wheat-grain and its various constituents in order to gauge the value of the bread or biscuit in such a diet. The results of these experiments are given in a separate communication* which contains references to the general literature of beri-beri, and to which the reader is also referred for a detailed description of the methods employed in the present work.

Polyneuritis of birds has been accepted as the complete analogy of beri-beri in man and, for the sake of brevity, we shall refer to the substance whose absence in a diet occasions polyneuritis in birds, or beri-beri in man, as the "anti-neuritic vitamine" (or anti-beri-beri vitamine). As regards the influence of high temperatures upon this substance, a great number of isolated observations exists in the literature, but no one hitherto seems to have attempted any systematic study of the point. Grijns (1901) showed that 1–2 hours' exposure to 120° C. destroyed the protective properties against polyneuritis of unmilled rice, "Katjang idjo" beans, and buffalo meat. Eijkman (1906) confirmed Grijns' results as far as unmilled rice was concerned, but did not succeed in destroying the anti-neuritic properties of horse-flesh by heating for two hours at 120° C. Schaumann (1910), however, induced polyneuritis in dogs with a diet of horse-flesh heated 1–3 hours at 120°–130° C., and Holst (1907) traced a loss of anti-neuritic vitamine in beef after heating to 110° C. for half-an-hour; this loss was much more marked after one hour at 120° C. In the case of dried peas or unpeeled barley, on the other hand; kept for half-an-hour at 115° C., no particular damage was done. Most observers have not detected any destruction of vitamine at 100° C.

In these and other experiments, the temperatures noted appear to be those registered by the autoclave or steamer used, no measurement being apparently made in the interior of the substance heated. This is an important point, as the latter temperature remains for a surprisingly long time far below the former (see Table I), especially if the material investigated has a low conductivity, which is true of most foodstuffs, especially if fairly dry. Further, no attempt has been made to investigate the time relations of the destruction process of the vitamine. This is obviously an exceedingly difficult problem, seeing that the only available instrument for measurement is so very imperfect. Nevertheless it seemed worth while to make the following effort.

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* The preceding paper.

Table I.—Curative Experiments showing the Influence of High Temperatures upon the Anti-neuritic (Anti-beri-beri) "Vitamine" contained in Wheat Embryo. Sample B, containing about 15 per cent. fine Bran. Water Content 11 to 14 per cent. Heating done in a steam autoclave. Material administered by the mouth as boluses made with a little water.

No. of expt.	Treatment of material.	Temperatures reached.	Time of heating.	Water content of material.	No. of bird.	Amount of dose given.		Result.	No. of days cure lasted.	Nature of symptoms.	Time of sickening.
						In terms of food-stuff.	In terms of dry weight.†				
		° C.	mins.	per cent.		grm.	grm.				days.
1*	Not heated, control birds	—	—	11	147	1·0	0·9	Improvement	3	Lameness	23
					161	1·0	0·9	Complete cure	5	Head symptoms	14
					162	1·0	0·9	No improvement, died	—	Lameness and crop-bound	17
					220	1·0	0·9	Died 24 hours later	—	Head symptoms and lameness	23
					221	1·0	0·9	Died 48 hours later	—	Lameness, head symptoms and crop-bound	23
					131	2·5	2·2	Complete cure	9	Lameness and head symptoms	24
					164	2·5	2·2	Very slight improvement for 24 hours	—	Lameness and crop-bound	26
					232	2·5	2·2	Died	—	Head symptoms	12
					234	2·5	2·2	Complete cure	9	Head symptoms	18
					236	2·5	2·2	Complete cure	8	Head symptoms	22
2	80 grm. placed in steamer at 100° C. for 60 mins.	90–100	40	13	132	2·5	2·2	Improvement	4	Lameness	31
					136	2·5	2·2	Incomplete cure	about 4	Head symptoms	21
					141	2·5	2·2	Complete cure	6	Head symptoms	18
					235	2·5	2·2	Cure	8	Lameness and head symptoms	17
					237	2·5	2·2	Died in 12 hours.	—	Lameness and head symptoms	18

3	40 grm. placed in steamer for 140 mins.	98–103	120	12	178	2·5	2·2	Complete cure	7	Head symptoms	14
					222	2·5	2·2	Incomplete cure	5	Head symptoms	22
					223	2·5	2·2	Complete cure	6	Head symptoms	22
					247	2·5	2·2	Complete cure	6	Head symptoms	18
					244	2·5	2·2	Great improvement	7	Lameness and head symptoms	18
4	100 grm. placed in autoclave at 113° C. for 60 mins.	102–107	40	12·2	142	2·5	2·2	Improvement	3	Lameness and head symptoms	18
					146	2·5	2·2	Complete cure	7	Head symptoms	18
					245	2·5	2·2	Incomplete cure	5	Lameness and head symptoms	21
					258	2·5	2·2	Cure	4	Head symptoms	18
					278	2·5	2·2	Cure	5	Head symptoms	15
5	100 grm. placed in autoclave at 122° C. for 60 mins.	110–117	40	14·4	160	2·5	2·1	No improvement, died in 48 hours	—	Lameness and crop-bound	16
					186	2·5	2·1	Very slight improvement	2	Lameness	10
					214	2·5	2·1	Great improvement, not cured	4	Head symptoms	20
					259	2·5	2·1	Died in 48 hours	—	Head symptoms	14
					276	2·5	2·1	Improvement, not a cure	4	Lameness and head symptoms	16
					145	5·0	4·3	Cure	10	Head symptoms	21
					246	5·0	4·3	Complete cure	5	Head symptoms	21
					256	5·0	4·3	Improvement	3	Head symptoms	15
					262	5·0	4·3	Improvement	7	Head symptoms and lameness	30
					257	5·0	4·3	Incomplete cure	4	Head symptoms	32
6	50 grm. placed in autoclave at 122° C. for 150 mins.	118–124	120	12·4	229	5·0	4·4	Improvement	5	Head symptoms	19
					230	5·0	4·4	Improvement	5	Lameness and head symptoms	19
					228	10·0	8·8	Slight improvement	5	Head symptoms	27
					281	10·0	8·8	Slight improvement	3	Head symptoms	17

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* The results of Experiment 1 have been used previously (see preceding paper) to institute a comparison between the curative values of wheat-germ and wheat-bran.

† After heating to 100–110° C.

Wheat embryo, in which the anti-beri-beri experiment is highly concentrated, was selected as suitable material for the present experiments, and 50–100 grm. were heated in a steam autoclave at different temperatures for different intervals of time. It was found that the interior of the material very slowly acquired the temperature of its surroundings, and special steps were taken to ascertain the exact temperatures experienced. In cases where the material was steamed at 100° C. (Expts. 2 and 3, Table I) the real internal temperature was measured by means of a maximum thermometer, whose bulb was inserted in the middle of the mass to be heated, and whose stem projected through the neck of the bottle containing it. The whole was lifted from the steamer for an instant at intervals of about 20 minutes, in order to obtain readings from the thermometer and to construct a curve of rise of temperature. In case of temperatures above 100° C. this procedure is not possible, and it was necessary to make special control experiments in which similar amounts of similar material were placed under exactly the same conditions as those of the actual test. Steam was blown off after various intervals of time from the start, the autoclave opened and note taken of the maximum temperatures registered both inside the vessel containing the wheat germ, and outside in the autoclave. By means of a series of such tests made separately, a temperature curve was constructed from which an accurate estimate could be made of the temperatures actually experienced by the material exposed. The data upon these points are set forth in the first three columns of Table I, above.

The experiments fall into two classes (*a*) those at or near 100° C., devised to be applicable to the ordinary processes of cooking, and (*b*) those above 100° C., intended to give information as to what may be expected to happen during sterilisation of tinned and canned foods. The effect on the properties of the wheat germ of exposure to these temperatures was studied by the following method. Comparison was made of the minimum doses of (*a*) original and (*b*) heated germ required to cure pigeons (of weight 300–400 grm.) acutely ill with polyneuritis, brought on by an exclusive diet of polished rice. From the results given in Table I it is seen that destruction of the anti-neuritic vitamine progresses very slowly at 100° C. or thereabouts. In fact, after two hours' exposure to such a temperature (Expt. 3) our experiments fail to reveal any significant loss in anti-neuritic properties—the curative dose of the heated "germ" remaining about 2·5 grm., the same as that of the unheated material (Expt. 1). After exposure for 40 minutes to a mean temperature of about 113° C. the efficiency, compared with the unheated control, is reduced to about one-half, and after subjection to 118°–124° C. for two hours, to something less than a quarter, possibly to one-tenth. The results in this series reveal all the imperfections alluded to elsewhere (see preceding paper) by the authors as peculiar to the curative type of experiment.

The material used in these experiments had rather a low water content, 10 per cent., and the vitamine present might be supposed to be protected from destruction on that score. A similar set of data are now being obtained, using a soluble yeast extract, containing over 60 per cent. water, whose vitamine content, reckoned on dry weight, is of about the same order as that of wheat germ. These experiments are not yet complete, but enough results have been already obtained to show that the extra water present during the heating has not enhanced at all the destructive effect of high temperatures upon the active substance.

It is therefore justifiable to apply the results obtained above with pure wheat germ to such processes as the baking of bread, biscuit, etc., where the water content may approach 50 per cent. The time taken to bake an average loaf does not exceed one hour, and the internal temperature rises to about 101° C. We may therefore conclude that, if wheat germ is included in the flour from which the bread is made, its anti-neuritic (anti-beri-beri) properties will survive the baking process, suffering very little damage.

The rapid destruction of anti-neuritic vitamine in the neighbourhood of 120° C. suggests that many tinned foods will be found deficient in respect of this constituent, and such we have found to be the case. We have examined two samples of army "meat and vegetable ration." In order to concentrate any vitamines they might contain, we extracted the air-dried material with alcohol (see Cooper, 1913, and H. C. and E. M. H., *loc. cit.*, p. 46), taking up the residue, after evaporating the alcohol under reduced pressure, with a small amount of water. In the case of Ration A, a very slight improvement in symptoms, but nothing approaching a cure, was obtained after administration of an extract equivalent to 350 grm. (112 grm. dry weight), and in case of Ration B, an incomplete cure after the equivalent of 440 grm. (106 grm. dry weight). Working with fresh meat, and using a similar method, Cooper (1913) obtained cures with the equivalent of 140 grm. (= 30 grm. dry weight). In the two preserved "rations" there would therefore appear to have been extensive destruction of anti-beri-beri properties during the process of manufacture.

Summary and Conclusions.

1. Exposure of wheat embryo to a temperature of about 100° C. for two hours resulted in no significant loss in anti-neuritic "vitamine." If, therefore, it is included in the flour from which bread or biscuit is made, it can be relied upon to retain its anti-neuritic properties after baking.

Table II.—Influence of High Temperatures upon the Anti-neuritic (Anti-beri-beri) "Vitamine" contained in Yeast Extract (Sample A, soluble; Water Content 65 per cent.). Curative experiments upon pigeons (300–400 grm.) suffering from acute polyneuritis, induced by an exclusive diet of polished rice. Material administered directly into the crop by means of a syringe with blunt nozzle. Heating done in a steam autoclave.

No. of expt.	Tempera-ture.	Time of heating.	No. of bird.	Amount of dose given.		Result.	Time taken by the bird to sicken.	Nature of symptoms.	Time the cure lasted.
				In c.c. of extract.	In grm. dry weight.				
	° C.	mins.					days		days
1	Not heated, control		294	2	0·7	No improvement, died in 3–4 hours	15	Sudden and very severe	—
			296	2	0·7	No improvement, died	21	Lameness and crop symptoms	—
			269	2	0·7	Great improvement	—	Lameness and head symptoms	3
			190	2	0·7	Incomplete cure	43	Lameness	6
			215	2	0·7	Complete cure	19	Head symptoms	6
			313	3	1·0	Improvement	12	Lameness	6
			200	3	1·0	Cure	17	Head symptoms	6
			201	3	1·0	Cure	17	Lameness	7
			293	4	1·4	Died	28	Bad case, crop involved	—
			192	4	1·4	Cure	22	Lameness and head symptoms	9
			189	4	1·4	Complete cure	15	Head symptoms	6
			193	4	1·4	Complete cure	24	Head symptoms	11
			299	4	1·4	Complete cure	20	Lameness	9
2	100	60	184	4	1·4	Died	21	Lameness, head and severe crop symptoms	—
			191	4	1·4	Improvement	14	Lameness and head symptoms	5
			187	4	1·4	Incomplete cure	25	Lameness	11
			188	4	1·4	Incomplete cure	25	Lameness and head symptoms	8
			185	4	1·4	Cure	21	Lameness and head symptoms	5

			280	6	2·1	Incomplete cure	18	Head symptoms	9
			303	6	2·1	Incomplete cure	21	Lameness and head symptoms	7
			203	6	2·1	Cure	22	Lameness and head symptoms	11
			227	6	2·1	Complete cure	13	Lameness and head symptoms	8
			301	6	2·1	Complete cure	16	Head symptoms	8
3	122	60	308	4	1·4	Improvement, but died after three days	30	Lameness and head symptoms	3
			306	4	1·4	Great improvement	25	Head symptoms	5
			307	4	1·4	Complete cure	19	Lameness and head symptoms	7
			194	5	1·7	Cure	15	Lameness	5
			195	5	1·7	Cure	21	Head symptoms	7
			163	6	2·1	Died	20	Lameness and crop symptoms	—
			305	6	2·1	Died	22	Lameness	—
			202	6	2·1	Cure	22	Lameness	7
			213	6	2·1	Cure	16	Head symptoms	5
4	122	120	319	6	2·1	Died in 12 hours	20	Head symptoms	—
			317	6	2·1	Died in 12 hours	20	Lameness and head symptoms	—
			310	6	2·1	Great improvement	31	Lameness and head symptoms	5
			312	6	2·1	Great improvement	30	Lameness	6
			297	6	2·1	Incomplete cure	—	Head symptoms	4
			322	10	3·5	Died in 24 hours	17	Head symptoms	—
			311	10	3·5	Cure	16	Lameness	5
			316	10	3·5	Cure	21	Head symptoms	9
			315	10	3·5	Complete cure	20	Lameness and head symptoms	8

2. At temperatures in the neighbourhood of 120° C., however, there was a swift destruction of anti-neuritic properties. This fact has an important bearing where diets are largely composed of preserved and tinned foods previously sterilised at temperatures above 100° C.

[*Addendum received August* 2, 1917.—Since the above paper was written the experiments with yeast extract alluded to on p. 65 have been completed and the results set forth in Table II, above.

The material used was a soluble yeast extract, made up to a solution of convenient strength; it contained about 35 per cent. of solids and 65 per cent. of water. As before, the heating was done in a steam autoclave; the solutions to be heated were placed in large-sized test-tubes, in quantities not exceeding 100 c.c. With this volume of liquid it was found that there was no significant delay in acquiring the temperature of the autoclave, if the test tube were placed in position while the latter was still cool.

The results obtained offer an interesting comparison with those of Table I above, where the substance heated (wheat germ) was comparatively dry (water content 10 to 14 per cent.). When due allowance is made for the large margin of error inherent in this type of experiment (see preceding paper, and p. 65, above), the results of Tables I and II are found to be in reasonable accord with one another. After exposure to 100° C. for one hour, slight loss of vitamine is evident in the yeast extract, a greater loss apparently than took place with the wheat embryo at this temperature, see Table I, Expts. 2 and 3. This may possibly be due to the excess of water contained in the extract. In the neighbourhood of 120° C. destruction of the vitamine was rapid in both cases.]

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Two Cases of Congenital Night-Blindness.

By Sir W. de W. Abney, K.C.B., F.R.S.

(Received July 21, 1917.)

In the recent communication of my paper on a "Fourth Sensation in Colour Vision," in April last, attention was called to the case of night-blind eyes as throwing light on the question of the functions of the rods and cones in the retina in regard to colour and colourless vision. I cited two cases of congenital stationary night-blindness, which, through the kindness of Mr. Nettleship, were brought to my laboratory for examination in view of certain researches in which Prof. (now Colonel) Watson and myself were together interested. As Colonel Watson has been long at the Front in France, and as he conducted a principal part of the only partially completed examination, I hesitated to give the details without his collaboration. I have now obtained his acquiescence in my request that I should communicate the results obtained to the Royal Society. I now do so. As a matter of fact, I had worked out the observations before my colleague left me for the Front some 2½ years ago, and these results I have put in the following communication :

I may say that any other form of night-blindness than those to which the late Mr. E. Nettleship introduced us would have been useless for the investigation on which we were engaged, as, if it were not so, night-blindness due to disease might call in question some of the deductions to be made. Mr. Nettleship's investigations of the family to which they belonged, and its history, left no doubt that we were dealing in our two cases with genuine cases of congenital stationary types.

The subject of congenital night-blindness (sometimes called moon-blindness) has been left with several obscure points unexplained, and the present communication, it is hoped, may throw light on some of them.

The late Mr. E. Nettleship collected a large number of pedigrees in which the characteristics of night-blindness are shown. His papers on the subject contain, it is believed, nearly everything that is worth knowing. The papers were published in the 'Royal London Ophthalmic Hospital Reports.'* He has amply proved that congenital night-blindness is hereditary. He commenced with the work that the late Florent Cunier† published in 1838 on a family in which night-blindness existed. Cunier gave long genealogical pedigrees with the names, domiciles, and dates of birth and marriages of the members. The total number of persons in seven generations was 629, of whom 56 were affected by night-blindness. By subsequent researches Mr. Nettleship was able to enlarge the pedigree, which as it stands now reaches 2121 persons, 1001 males and 960 females (of the remainder, sex not known), of which 72 males and 62 females, and one (sex not stated) are known to have been night-blind, and all of whom sprang from a common ancestor, Nougaret by name, who lived about 1600.

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* See vol. 17, Part III, and vol. 27 of the 'Ophthalmic Society's Transactions,' besides others.

† Fl. Cunier, 'Médecin Militaire,' "Histoire d'une Héméracopie Héréditaire depuis Deux Siècles dans une Famille de Vendemair près Montpellier."

This is only one of the several family pedigrees which Mr. Nettleship gives, but all show that there is heredity in congenital night-blindness. There is a great distinction between the night-blind and the congenital monochromatic vision, of which cases are rare. The one can see in daylight all the spectrum colours as ordinary normal vision does, the other does not. A quotation from one of Mr. Nettleship's papers will give an idea of how congenital night-blindness manifests itself. After a description of the retina and refraction, he says: "(The patient) is extremely night-blind, but can see some of the brighter stars, and can do well by bright moonlight and artificial light. On a moonless night has a great difficulty in finding his way, cannot see the street before him, but guides himself in keeping to the middle of the road, and looking up and recognising the sharp and black line of the house tops against the sky.

"A poor man, vine labourer, slow and awkward by training, but intelligent and most graphic in his description if allowed time to find words and gestures. In walking up and down the partially darkened room" (in which he was examined), "he would put his hands out and sometimes stop altogether; indeed, he had the aspect of one blindfolded. He was quite unable to see the fingers or even the back of the hands at 0·3 metre with an illumination that was ample for a normal person. He had been like this as long as he could remember (he was 46 years old), but had, nevertheless, been obliged to serve five years in the (French) Army. His sight is not getting worse.

"This man's son, 16 years old, is as night-blind as his father."

In the case of the monochromatic vision to which I have alluded, they all saw well in low illumination, and but moderately in bright daylight. These cases have almost exactly opposite kind of vision. For this reason a physical examination of the vision of the night-blind seems desirable, and though this examination was not completed in some points such as colour field, it is believed that we have indications of marked differences in the perception of colours and light when tested by the spectrum method.

The clinical aspects of night-blindness, of course, my colleague and myself are unable to discuss. It suffices to say, I think, that these two cases fulfilled the object we had in view, viz., to examine two of the congenital cases which (the late) Mr. Nettleship included in one of his pedigrees.

The first patient, Mr. E., a clergyman, came to the laboratory on February 25, 1913, when he was examined in the writer's apparatus and presence for his extinction of colour from the red to the blue of the arc spectrum.

The luminosity of the D line coming through slits to the screen was the standard of the intensity of the luminosity of the different rays of the spectrum. This was gradually diminished by an annulus placed in the path of the ray until he just saw no light in the illuminated small square in the darkened camera attached to the spectroscopic apparatus. He said that all light had vanished when the colour was extinguished. He repeated the observations in a reverse manner, noting the advent from darkness of the spectrum colour.

This series of observations was repeated in a second apparatus in which the source of light was a Nernst lamp, the thread of which was rendered incandescent by an ampère of current from a battery of 100 volts; the current was kept constant. Similar observations were made by this apparatus and recorded, and afterwards converted to the arc scale. Finally, the luminosity curve of his spectrum was taken and compared at the time with that of my colleague.

In his observations he invariably said that, when the extinction was noted by the reduction of the said intensity, whenever the colour was gone all the light had also gone. In other words, the same reduction in intensity of the light was the threshold for both light and colour. In the paper, "The Threshold of Vision for Different Coloured Lights,"* the same identity of extinctions of light and colour at the fovea of Class I retina is to be noted, as given by all observers, though the extinction of light outside the fovea is nearly 0·0001 times less than it is for colour at the fovea.

Comparing the loss of colour for my own eye (Class II) when the intensity of the spectrum D is one candle-foot, the colour is extinguished at an intensity of about 0·0016 candle-foot, and the extinction of light at about 0·000035 candle-foot intensity. For "E." the extinction of both light and colour in both cases takes place at about 0·0015–6. This indicates that the extinction of the feeble white light (or, as I have called it, of the fourth colourless sensation) is dependent on some other retinal perception that the normal eye possesses beyond that possessed by the night-blind. The same is probably the cause of the difference in the fovea of No. I retina compared with that of No. II retina. If the rods and cones fill the places in reference to the light sensations which have been allotted to them, as stated in my last communication on the fourth sensation, then there is an absence of sensitive rods in the whole retinæ of the night-blind. The same remarks may apply to the observer B. and his measures, though they differ slightly in shape for those of E., but not more so than do the curves of various observers in the 'Philosophical Transactions' paper just referred to.

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* 'Phil. Trans.,' A, vol. 216.

On May 20, 1913, B. came for his examination at the colour laboratory. He is a clergyman, and is, we believe, a cousin of E. His examination was conducted on the same lines as that of E., the arc light alone being used. In addition, he was made to match the colour of the D sodium line with mixed colours of thallium and lithium blue, in what is called the anomaloscope, a very useful instrument, though only partially indicative of any defect in colour vision.

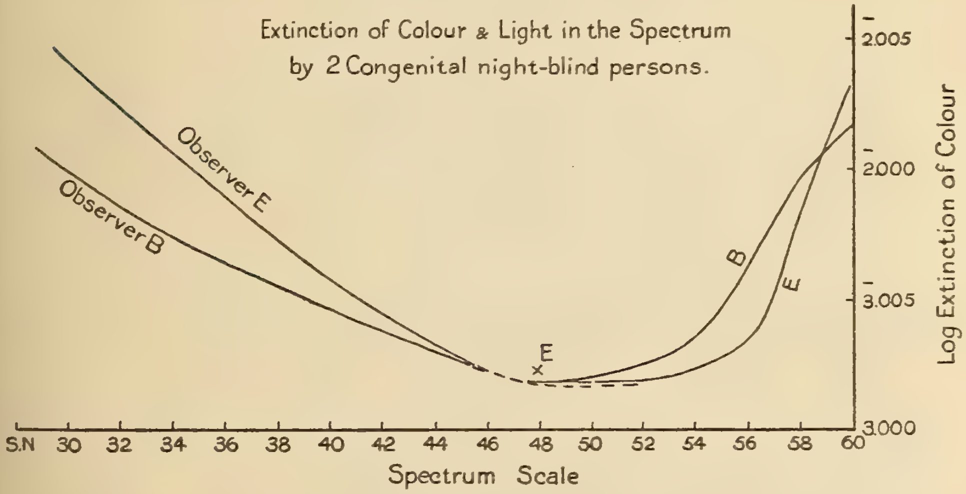
[In Colonel Watson's and my paper, jointly published in the 'Philosophical Transactions,' it was shown that there seemed to be two classes of retina: one in which only the colours of the spectrum were recognised in the spectrum and no colourless rays affected the fovea, and the other class in which both colour and the feeble colourless rays were equally effective. As we soon gathered the retinæ of the two patients at any part apparently were not stimulated by the colourless rays, we determined to compare them with the No. I class of retina, for which purpose we had to reduce the arc results into the Nernst coloured spectrum. This was done, and the night-blinds' extinction of colour coincided practically with the threshold of No. I retina at the fovea.]

It should be mentioned that Mr. Nettleship divides congenital night-blindness into two classes, both showing the same night-blindness. The one is myopic, but the other apparently normal, as far as refraction is concerned. The two observers appear to be in the first division.

The night-blind luminosity curves, taken with a luminous spectrum of the arc, compared with that of the normal eye, show that the intensity of colour is the same for both. Allowing for the difficulties of the shadow test, and a certain error in observation which is found in the flicker test, the curves of luminosity of both may be said to be the same. The luminosity curves can be best compared by making the night-blind measures the numerator and the normal measure the denominator of a fraction. The following are the fractions for E. and B.:—

S.S.N.	58.	56.	54.	52.	50.	48.	46.	44.	42.	40.	38.	36.	34.
$\frac{E}{N}$	1·12	1·12	1·06	1·00	1·00	0·98	1·00	0·92	0·90	0·93	0·96	1·02	1·05
$\frac{B}{N}$	0·97	1·00	1·00	1·02	1·02	1·00	1·03	1·08	1·08	1·08	1·10	0·94	1·05

Allowance has to be made for a difference in pigmentation of the foveal region. It has been shown in my recent communication on the fourth sensation that the added luminosity of the sensation of this fourth sensation is



negligible when the spectrum is of normal brightness (say when the D ray is of ½ candle-power brightness at foot distance from the screen). These measures show that the luminosity of such a spectrum is, within the limits of error, the same in both cases.

Annulus 315. Observer E.

S.S.N.	Corrected annulus reading.	Absorption factor.	Product of 2 and 3.	Or	Reduction to one candle foot + $\bar{1}$·155 (log.)	Limit of vision.
60	66	−0·01170	−0·772	$\bar{1}$·228	$\bar{2}$·383	0·0242
58	106	−0·01184	−1·255	$\bar{2}$·745	$\bar{3}$·900	0·0079
56	143	−0·01197	−1·712	$\bar{2}$·288	$\bar{3}$·440	0·00276
54	158	−0·01212	−1·915	$\bar{2}$·085	$\bar{3}$·240	0·00174
52	153	−0·01229	−1·979	$\bar{2}$·021	$\bar{3}$·176	0·00150
50	124	−0·01244	−1·966	$\bar{2}$·033	$\bar{3}$·188	0·00154
48	136	−0·01259	−1·926	$\bar{2}$·074	$\bar{3}$·229	0·00170
46	144	−0·01273	−1·833	$\bar{2}$·167	$\bar{3}$·322	0·00210
44	136	−0·01288	−1·752	$\bar{2}$·248	$\bar{3}$·403	0·00253
42	126	−0·01302	−1·641	$\bar{2}$·359	$\bar{3}$·514	0·00374
40	115	−0·01318	−1·516	$\bar{2}$·484	$\bar{3}$·639	0·00436
38	105	−0·01334	−1·401	$\bar{2}$·599	$\bar{3}$·754	0·00560
36	91	−0·01354	−1·232	$\bar{2}$·768	$\bar{3}$·923	0·00838
34	78	−0·01374	−1·072	$\bar{2}$·928	$\bar{2}$·083	0·0121
32	66	−0·01390	−0·917	$\bar{1}$·088	$\bar{2}$·238	0·0173

Annulus 315. Observer B.

S.S.N.	Corrected annulus reading.	Obstruction factor.	Product of 2 and 3.	Or	Reduction of D to one candle foot + $\bar{1}$·155 (log.)	Limit of vision.
60	73	−0·01170	−1·088	$\bar{2}$·912	$\bar{2}$·097	0·00125
58	102	−0·01184	−1·208	$\bar{2}$·792	$\bar{3}$·947	0·00807
56	135	−0·01197	−1·156	$\bar{2}$·484	$\bar{3}$ 639	0·00436
54	147	−0·01212	−1·782	$\bar{2}$·218	$\bar{3}$·373	0·00236
52	155	−0·01229	−1·905	$\bar{2}$·095	$\bar{3}$·250	0·00175
50	157	−0·01244	−1·953	$\bar{2}$·047	$\bar{3}$·202	0·00159
48	157	−0·01259	−1·972	$\bar{2}$·028	$\bar{3}$·183	0·00152
46	152	−0·01273	−1·935	$\bar{2}$·065	$\bar{3}$·220	0·00166
44	144	−0·01288	−1·855	$\bar{2}$·145	$\bar{3}$·300	0·00200
42	136	−0·01302	−1·771	$\bar{2}$·229	$\bar{3}$·384	0·00242
40	128	−0·01318	−1·687	$\bar{2}$·313	$\bar{3}$·468	0·00294
38	120	−0·01334	−1·601	$\bar{2}$·394	$\bar{3}$·549	0·00354
36	112	−0·01354	−1·516	$\bar{2}$·484	$\bar{3}$·639	0·00436
34	103	−0·01374	−1·415	$\bar{2}$·585	$\bar{3}$·740	0·00550
32	93	−0·01390	−1·293	$\bar{2}$·707	$\bar{3}$·862	0·00727

B, 61·3; Li, 59·8; C, 58·1; D, 50·6; E, 39·8; *b*, 37·7; F, 30·05; Blue Li, 22·8; G, 11·2. The above give the positions of Fraunhofer lines on the (arc) spectrum scale.

PROCEEDINGS
OF
THE ROYAL SOCIETY.

Series B. Vol. 90. No. B 625.

BIOLOGICAL SCIENCES.

CONTENTS.

Page

The Enzymes Concerned in the Decomposition of Glucose and Mannitol by Bacillus coli communis. Part II.—Experiments of Short Duration with an Emulsion of the Organisms. By EGERTON CHARLES GREY (Beit Memorial Research Fellow) 75

The Enzymes Concerned in the Decomposition of Glucose and Mannitol by Bacillus coli communis. Part III.—Various Phases in the Decomposition of Glucose by an Emulsion of the Organisms. By EGERTON CHARLES GREY (Beit Memorial Research Fellow) 92

The Germicidal Action of Ultra-violet Radiation and its Correlation with Selective Absorption.—Addendum. By C. H. BROWNING, M.D., and SIDNEY RUSS, D.Sc. 107

PRINTED FOR THE ROYAL SOCIETY AND SOLD BY
HARRISON & SONS, 45, ST. MARTIN'S LANE, LONDON, W.C.
Price One Shilling and Sixpence.

No. B 625.

January 1, 1918.

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The Enzymes Concerned in the Decomposition of Glucose and Mannitol by Bacillus coli communis. Part II.—*Experiments of Short Duration with an Emulsion of the Organisms.*[*]

By Egerton Charles Grey (Beit Memorial Research Fellow).

(Communicated by Dr. A. Harden, F.R.S. Received July 25, 1917.)

(From the Laboratory of Prof. A. Fernbach, Institut Pasteur, Paris.)

CONTENTS.

	PAGE
Bacteriological Technique	76
Apparatus for the Study of a Complete Fermentation	77
Chemical Technique	80
The Fermentation of Glucose in the Presence of Chalk by an Emulsion of *B. coli communis*	83
The Comparison between the Products from Glucose and those from Mannitol	89
Summary and Conclusions of Part II	91

The earlier experiments described by the writer upon the decomposition of glucose and mannitol by *B. coli communis*[†] were open to the objection from the biological side that no attempt was made to distinguish those products which arose by enzyme action from those which might be more particularly associated in their formation with the growth and multiplication of the cells, and from the chemical side that certain substances of unknown composition, such as peptone, were employed, so that it was not possible to be sure that some of the products of fermentation had not been derived from this source.

To overcome these objections the author has adopted a new plan of work which aims at separating the process of growth from that of fermentation.

The method consists in growing the bacteria upon a suitable medium and adding them when sufficiently developed to a solution of the substance to be fermented. The bacteria are washed from the surface of the medium by means of a solution of potassium sulphate, and the emulsion so obtained is added to the substance to be fermented, in the presence of chalk. Under such circumstances an amount of bacteria which would weigh, when dry, 1 grm. will, in the case of *B. coli communis*, bring about the degradation of 40 grm. of glucose in 48 hours.

A period of 48 hours is too long for the fermentation if it be desired to study the separate phases of the fermentation process, nevertheless, in this communication a series of experiments will be described wherein the duration of each experiment was of this order, since, though these results represent the average of several fermentation processes which occur together, they have the value of indicating the manner in which the various products of the fermentations vary with changes in the conditions of the experiment and they give information as to the probable origin of succinic acid in this particular fermentation. In Part III an experiment will be described in which the several phases which characterise this bacterial fermentation process have, to a certain extent, been separately studied.

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[*] Part I of this work appeared in these Proceedings, B, vol. 87, p. 472 (1914).

[†] Grey, E. C., 'Roy. Soc. Proc.,' B, vol. 87, p. 472 (1914).

Bacteriological Technique.

For the sake of future reference, and for present uniformity, it has been thought best to employ only standard organisms. In these experiments the *B. coli communis* of the collection of the Institut Pasteur has been used. The treatment of the organism prior to its mixture with the substance to be fermented has also been made to conform to a uniform plan. In each case a loopful taken from a growth on agar has been inoculated into beef bouillon and the fluid incubated for 24 hours at 38° C. This culture has been used to inoculate a series of Roux bottles containing agar prepared after the manner recommended by Dr. Martin. The technique of inoculating the bottles, as well as for the subsequent removal of the growth, is practically that employed by Dr. Salambini, of the Pasteur Institute. Instead of the saline solution employed for the preparation of vaccines a solution of potassium sulphate or mixture of this and magnesium sulphate is employed, as it is not desirable to introduce the salt of any volatile acid such as hydrochloric acid into the fermentation solution. The growth in the Roux bottles has been allowed to take place during 48 hours in each case. At the end of this period about 100 c.c. of sterile sulphate solution is introduced into each bottle. The concentration of the sulphate solution used depends upon the object of the experiment. A very great variation in concentration is permissible. The maximum decomposition of glucose obtained in a series of preliminary experiments was found to occur with a concentration of potassium sulphate equivalent to $3N/40\ K_2SO_4$. With this concentration of potassium sulphate in the fermentation solution 1 grm. of bacteria decomposed 40 grm. of glucose in 48 hours. The only ion which it was found of value to add to the potassium sulphate solution was magnesium. The addition of sulphate of magnesium will increase the rate of fermentation by about 15 per cent. With the exception of calcium, which the writer has found to be of benefit in the decomposition of sugar by *B. coli communis,* and which is present in excess in these experiments, no other metallic ion nor any negative ion was found to assist the fermentation when added to the emulsion of bacteria washed off the surface of the agar. Such ions if necessary are required only in the traces in which they exist in the emulsion so obtained.

The metallic ions added were those of manganese, iron, zinc and aluminium; the negative ions, chloride, nitrate, silicate and phosphate.

The writer has found that in order to grow *B. coli communis* on an artificial medium such as a solution of sugar and mineral sulphates together with some source of nitrogen such as ammonium sulphate or an amino-acid (asparagine, alanine, glycine) it is necessary or at least highly beneficial to add a phosphate; nevertheless no beneficial effect was obtained upon the rate of fermentation when phosphates were added to the fully grown organisms.

A solution of 6 grm. of potassium sulphate and 0·5 grm. of magnesium sulphate per litre is suitable for the study of the decomposition of substances allied to glucose under the influence of *B. coli communis.*

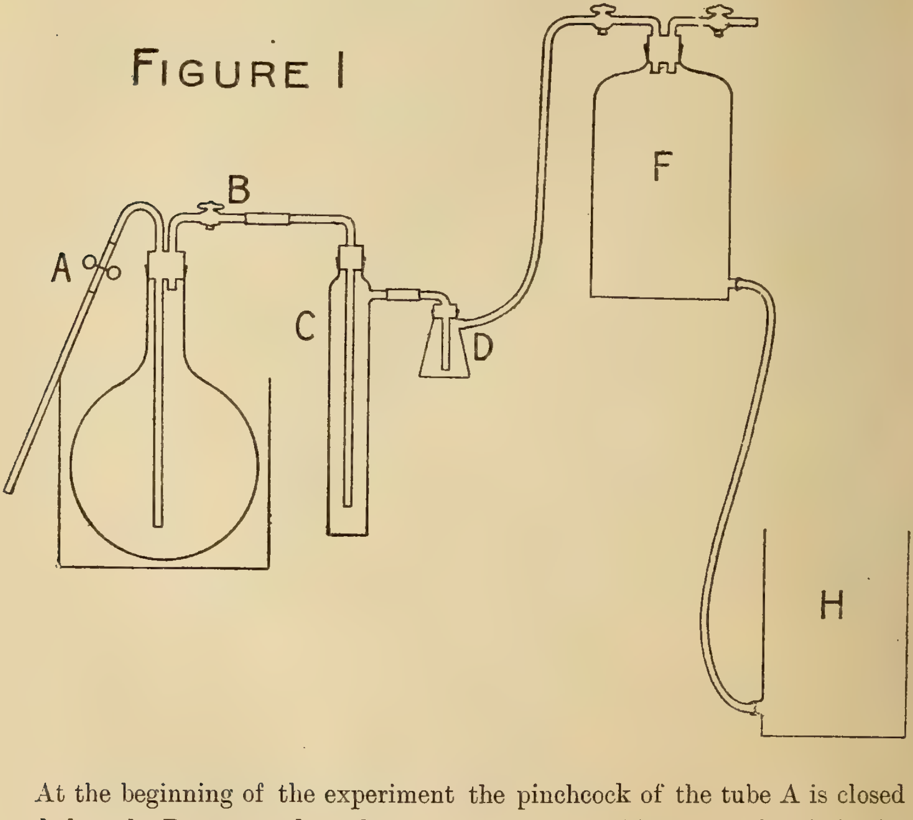
If each of the Roux bottles contain about 100 c.c. of agar and the growth be obtained in the manner described, it will be found that 10 bottles will yield about 1 grm. of dried bacilli. The amount of organic matter outside the bodies of the bacteria which is washed off the agar with the potassium sulphate solution has been found to be fairly constantly equal to three times the weight of the yield of dried bacteria. The weights of dried bacteria and soluble organic matter are determined for each experiment by centrifugalising a sample of the emulsion and determining the weight of bacteria directly, and the organic matter in the solution by evaporation in a platinum vessel and weighing after drying at 110° C. and after incinerating, or the bacteria may be determined indirectly by evaporating to dryness two separate samples, from one of which the bacteria have been separated by the centrifuge. The difference in weight represents the weight of bacteria in the sample of emulsion.

Arrangement of Apparatus for the Study of a Complete Fermentation.

The fermentation flask rests in the water-bath, regulated for the required temperature. The flask has a capacity of about 2 litres. The volume of the fermentation solution at the beginning of the experiment is about 1½ litres. At the end of the experiment water is introduced to displace the gas above the solution in the flask.

In the experiments described below the procedure adopted was as follows: The flask containing chalk and the substance to be fermented dissolved in about 800 c.c. of distilled water was sterilised by heat. The solution was cooled to 38° C. and the emulsion of bacteria introduced, the amount added being determined by weighing the flask before and after the introduction of the emulsion. The flask was sterilised while plugged with cotton wool, and after the introduction of the bacteria the cotton wool plug was replaced by the stopper seen in the figure. The practice has always been adopted of either sterilising the stopper separately by steam or flaming the tubes and rubber previous to its introduction into the flask, but it is almost certain that this precaution is unnecessary, since the size of the population of bacteria introduced at the beginning of the experiment precludes the possibility of the results being appreciably influenced by subsequent contamination even should such occur. This is one of the great advantages of the new technique.

FIGURE I



At the beginning of the experiment the pinchcock of the tube A is closed and the tube B connected to the water-pump. By this means the air in the flask is exhausted, the solution being brought to the boil under the reduced pressure. The tap B is then closed and, the tube A having been put into communication with a reservoir of nitrogen, the pinchcock is opened and nitrogen allowed to enter to replace the air which has been removed. The operation is repeated a second and third time to ensure complete removal of oxygen from the flask either in the gas space or dissolved in the solution.

The nitrogen which enters through A is filtered through a plug of cotton wool.

The tube B is in connection with a wash-bottle C of 500 c.c. capacity, which contains a solution of sodium hydroxide free from CO_2. If the strength of the solution is about normal, it is found that the one bottle of this capacity suffices to absorb the carbon dioxide from the evolved gases although these may pass through the solution at a considerable rate. A second small bottle D has been introduced for safety; it contains about 100 c.c. of the sodium hydroxide solution. The mixture of nitrogen introduced at the beginning of the experiment and the hydrogen evolved from the fermentation is collected in the reservoir F. By lowering the vessel H the difference of level between F and H may be adjusted to counteract the weight of the column of sodium hydroxide solution which has to be lifted by the gases issuing from the fermentation flask. The gases are thus evolved under atmospheric pressure. The method of collecting the gases here described is much simpler than that in which they are collected in an evacuated flask over mercury. As far as the estimation of CO_2 is concerned the method is just as accurate, but the estimation of hydrogen is not so accurate owing to the difficulty of accurately graduating a vessel of the form F and also because of the solubility of hydrogen in water.

But apart from its simplicity the present method has now been adopted because the rate of evolution of the gases is too great to permit of them being collected through a column of mercury into a vacuum even if the wide form of tube recommended by the writer in an earlier communication be adopted. It may be pointed out, however, that a combination of the sodium hydroxide absorption bottles with the mercury collecting apparatus employed by Harden would be still better than the system here employed if an accurate estimation of small amounts of gases other than carbon dioxide was to be made. The gases in this case would, after passing through the sodium hydroxide solution, pass on into the mercury gas-collecting apparatus, the size of which could be considerably reduced.

A further point to be noted in connection with the form of apparatus now employed is that it is suitable for the study of aërobic as well as anaërobic fermentation, for oxygen can be readily admitted during the course of the experiment by opening the pinchcock A.

In the series of experiments described below, the practice has been adopted of stopping the fermentation at the time desired by raising the temperature of the water-bath to about 70° C. and maintaining it at that temperature for about half-an-hour. At the same time hot distilled water has been introduced to displace the gases above the solution in the flask.

By this operation not only is the reaction of fermentation brought to an end and the solution made practically sterile, but a large part of the dissolved carbon dioxide is evolved, so that it is possible to estimate the carbon dioxide which remains in the solution when cold more accurately than when the solution was saturated as in the older experiments.

Chemical Technique.

Where no particular method is indicated it will be understood that the methods employed by Harden* and already described have been adhered to.

The Estimation of Carbon.—It is sometimes of great help in following the course of the fermentation to be able to estimate in a simple manner the carbon dissolved in the solution. This can be done by the volumetric method described by the writer.†

In cases where the fermentation was interrupted before the whole of the sugar was fermented an estimation of carbon was made in the solution after the removal of all the products known to be formed, but in no case was any appreciable amount of carbon found in excess of that which corresponds to the sugar present. From this it was concluded that if any glycerine had been left over during the fermentation it was in amount too small to be taken into account. The estimation serves as a check that the whole of the products have been accounted for.

Carbon Dioxide.—The carbon dioxide which remains dissolved in the fermentation solution is estimated by removing a sample with a pipette and mixing it with a solution of standard barium hydroxide. This is permissible in the case when the solution is not saturated with gas, as in the experiments described in Part II, but when the concentration of dissolved gas is greater the more accurate method described in Part III is to be recommended.

The method of collecting the evolved carbon dioxide has been described. The sodium hydroxide used for the absorption is prepared as follows: A known volume of a strong solution of sodium hydroxide is placed in a tall cylinder and a sample of the solution used to determine the amount of carbon dioxide which it contains. Sufficient standard barium hydroxide is added to completely precipitate the whole of the CO_2 and the precipitate allowed to settle. The clear fluid is siphoned into a large volume of water which has been previously freed from CO_2 by a rapid current of CO_2-free air.

The estimation of the amount of carbon dioxide in the original sodium hydroxide solution employed may be made in the apparatus described for the estimation of carbon. In the absence of such an apparatus an accurate method is to mix the sample with acid and carry off the disengaged gas in a current of CO_2-free air (the CO_2 produced in the experiment being absorbed in standard barium hydroxide). When once a solution free from CO_2 has been obtained all the subsequent estimations of CO_2 are simple titrations.

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* Harden, A., 'Chem. Soc. Journ.,' 1901, p. 610; Grey, E. C., 'Roy. Soc. Proc.,' B 87, p. 472 (1914).

† Grey, E. C., 'Chem. Soc. Journ.,' 1914.

To determine the amount of carbon dioxide evolved during the fermentation, the sodium hydroxide solution of the bottles C and D is transferred to a graduated flask and diluted to a definite volume. Of this solution a sample is mixed with standard barium hydroxide solution, and after filtration from the precipitate of barium carbonate the diminution of alkalinity is determined by titration with standard acid. An example of an estimation may be given.

250 c.c. of the CO_2-free sodium hydroxide solution was diluted to 1 litre. Of this solution 20 c.c. corresponded to 54·88 c.c. of N/10 H_2SO_4.

Another 250 c.c. of the same solution was diluted with water in the bottles C and D for the absorption of the CO_2 evolved in an experiment, and the solution was ultimately diluted to 1 litre; of this solution 20 c.c. was mixed with 50 c.c. of standard barium hydroxide solution and the mixture filtered; 20 c.c. of the filtrate neutralised 33·50 c.c. of N/10 H_2SO_4.

20 c.c. of the diluted NaOH corresponds to	54·88 N/10 H_2SO_4
50 c.c. of barium hydroxide corresponds to	102·80 N/10 H_2SO_4
	157·68
Alkalinity after absorption 33·5 × 7/2	= 117·25
Carbon dioxide in 20 c.c. of NaOH solution	= 40·43 N/10

Total carbon dioxide evolved 202·15 c.c. normal.

The Estimation of Alcohol.—The most accurate and at the same time the simplest method of estimating alcohol is that of Martin.* According to the original method the fermented solution is distilled directly into the mixture of bichromate and sulphuric acid, but this cannot be done in the presence of acids which could themselves reduce the bichromate. A previous distillation from an acid solution must be followed by a distillation from an alkaline solution. It is not necessary to distil the alcohol directly into the bichromate mixture, but the dilute solution of alcohol may be added gradually from a pipette to the oxidising mixture and the solution maintained at the temperature of the water-bath for 10 minutes. The excess of bichromate is titrated with ferrous sulphate solution in the usual way.

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* Martin, 'Mon. Sci. Quesn.,' 1904.

Volatile Acids.—Under certain circumstances it has been found that the reduction of mercuric chloride to mercurous chloride in the estimation of formic acid may be accompanied by a blackening indicative of further reduction. It was found that under such circumstances the solution of the sodium salts of the volatile acids obtained from the fermentation gave a precipitate of iodoform when treated in the cold with sodium hydroxide and iodine solution.

The fact that the production of iodoform in the cold occurred after the solution of the sodium salts of the volatile acids had been concentrated from a volume of 2 litres to 200 c.c., and also that the distillate obtained for the estimation of alcohol did not give the reaction, shows that the reducing substance is an acid, and it calls to mind the formation of pyruvic acid observed by Fernbach and Schoen* under the same circumstances from yeast. In this case the amount of acid is very small and no conclusive reaction could be obtained to decide as to its nature.

The Separation of Succinic and Lactic Acids.—The details to which it is necessary to adhere in order that the Pasteur method of separating succinic acid from other acids may yield accurate results have been described by the writer elsewhere.†

An important point to note in connection with the present technique is that the absence of peptone obviates the difficulty which was experienced in the earlier experiments in the examination of the residual solution after the distillation of the volatile acids. Also during the distillation itself there is here none of the objectionable frothing to which solutions of peptone may give rise. Chiefly, however, is it essential that there should be no peptone in the solution which is used for the separation of succinic and lactic acids, as has been pointed out in the special communication referred to.

The Estimation of Residual Carbohydrate or Allied Substance in the Solution after the Fermentation.—Up to the present the number of substances which could be studied as to the decomposition products resulting from their fermentation has been limited by the difficulty of estimating the portion which remained unfermented after the experiment. It was partly for this reason that the writer introduced the volumetric method of estimating carbon. Even in the case of glucose it may not always be safe to rely upon the figure for the residual portion as indicated by the reduction of copper solution, and certainly the reduction should be determined also after the residual solution (in which the glucose is to be determined) has been hydrolysed by acid, as will be seen from the experiments described in Part III. But with the method of studying the fermentation now employed there is a very simple method of getting over the difficulties which might arise from the presence of residual unfermented substance difficult to estimate. It consists in so adjusting the weight of bacteria employed that, in the time during which the experiment is to be continued, the whole of the substance to be examined will be fermented. It is thus possible to control the fermentation in a way which was not possible in the earlier experiments and it should be possible to study the fermentation of carbohydrate and allied substances for which no methods are at hand for their estimation with accuracy, as, for example, the various polyhydric alcohols. The method has been used here for the comparison of the products from mannitol with those from glucose under the same conditions.

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* Fernbach and Schoen, 'Comptes Rendus,' vol. 157, p. 1478 (1913); *ibid.*, vol. 158, p. 1719 (1914).

† Grey, E. C., 'Bull. Soc. Chim.,' 1917; 'Biochem. Journ.,' vol. 11, p. 2 (1917).

The Neutralisation of the Medium by Chalk.—Even if the fermentation flask be repeatedly shaken during the course of the fermentation, there will be periods during which the chalk will settle to the bottom of the flask if, as in the usual experiment, the fermentation be continued overnight without agitation. During such periods the acidity may increase sufficiently to impede or alter the course of a fermentation. The writer has employed a very simple device for preventing the settling of the chalk during the experiment. It consists in introducing the chalk into small sacks of cloth, in each of which is placed also a small piece of cork. The weights of chalk and cork are so balanced that the sacks just sink to the bottom of the flask when they are first introduced; during the course of the fermentation the acid disengaged penetrates the sacks and they become swollen with gas and rise to the top of the solution. During this movement up and down the chalk contained in the sacks becomes gradually liberated, so that the solution is always turbid with chalk without being mechanically agitated from without.

The Fermentation of Glucose in the Presence of Chalk by an Emulsion of B. coli communis.—A series of experiments will be now described in which an emulsion of *B. coli communis* was allowed to act for a period of 48 to 68 hours on a solution of glucose in distilled water, containing varying quantities of potassium sulphate. The fermentations took place in the presence of chalk.

An accidental rise in the temperature during the early stage of one of the first experiments (No. 3) gave rise to a phenomenon which has not since been repeated to the same extent. The bacteria introduced into the fermentation flask contained 0·5 grm. of fatty or waxy material. At the end of the experiment the weight of this material was 3·5 grm. There had been thus a synthesis of 3 grm. of a material which, under normal circumstances, does not accumulate. On the contrary, in most experiments the amount of fatty material in the bacterial cells appears to be used up during the fermentation.

It is to the attempt to understand the cause of this synthesis of fat or wax (which was accompanied by a high yield of lactic acid) that the series of experiments described in Part II owes its length. Attempts were made to obtain a similar yield of the substance by change of the temperature in various ways, and by alteration in the concentration of the salt. The attempts were never as successful as in Experiment 3, but the variations in the resulting proportions of the products led to the belief that the fermentation by *B. coli communis* really represented the sum of several independent fermentations, which belief proved to be amply justified by the results of the experiment which was devised to settle this question, and which is described in Part III.

In Table I the results are arranged in order of decreasing yield of alcohol. With the method of Martin, the estimation of alcohol is of a high order of accuracy. In fig. 2 these results are recorded graphically. In fig. 3 the same results are arranged from left to right, in order of increasing concentration of the potassium sulphate used in the experiments. It will be seen that, with the exception of the relative positions of No. 3 and No. 5, the results grouped in this way give rise to much the same curves as when arranged according to the yield of alcohol, from which it may be inferred that the cause responsible for the variations in the proportions of the products is the change in the concentration of the salt solution. The manner in which the change in the concentration of the potassium sulphate solution influences the proportion in which the products of the fermentation appear at the end of the experiment was not clear until the experiments described in Part III had been carried out, for, unfortunately, the bacteria were not counted in the earlier experiments. It is now clear, however, that in dilute solution there is a greater diminution in the number of living bacteria, or a greater action of the dying or dead cells, than in the cases where the concentration of potassium sulphate is greater, and, as will be seen later, the death of the cells is accompanied by the production of alcohol and those other products which are formed in conjunction with it, while the production of lactic acid is more closely associated with the rapid multiplication of the cells. Thus, at the one end of the figures the results are to be correlated with rapid death of the bacteria introduced, and at the other with less death and more multiplication.

Note.—In fig. 3 Experiment 3 has been placed at the end of the series. There was uncertainty about the conditions of this experiment. It, however,

Table I.—Collected Results of the Action of *B. coli communis* upon Glucose. The results are expressed as percentages upon the sugar consumed. The numbers refer to the original order in which the experiments were carried out.

	No. 7.	No. 4.	No. 6.	No. 8.	No. 10.	No. 11.	No. 3.	No. 5.
Carbon dioxide	19·38 } 24·60	21·25 } 25·13	16·00 } 21·42	22·46 } 25·65	15·24 } 27·53	14·57 } 20·75	16·50 } 24·00	11·90 } 20·92
Formic acid	5·22	3·88	5·42	3·19	12·29	6·18	7·50	9·02
Acetic acid	18·56	15·64	16·02	17·99	22·02	19·89	9·67	23·23
Lactic acid	16·93	21·25	20·05	19·99	25·46	36·52	49·79	27·09
Succinic acid	17·36	19·59	18·01	15·05	11·23	9·76	3·97	10·69
Alcohol	20·06	16·63	15·02	14·28	13·36	13·17	10·63	8·31
Hydrogen	0·35	—	0·35	0·26	0·21	—	—	0·26
Total	97·86	98·24	92·97	93·22	99·81	100·09	98·06	90·50
Ratio CO_2/H_2	1·25	—	1·05	1·96	1·78	—	—	1·03

Where a blank has been left hydrogen was not estimated.

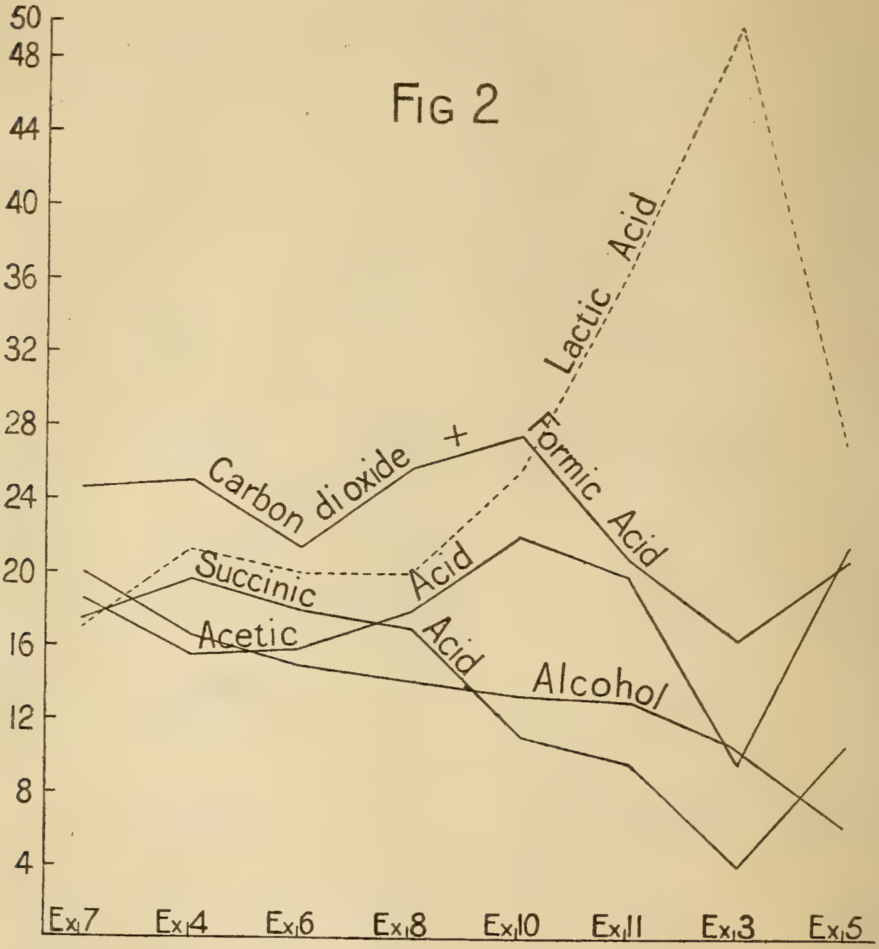
Appendix to Table I.—Data as to the Conditions of the Experiments recorded in Table I.

	4.	7.	8.	6.	10.	5.	11.	3.
Concentration of K_2SO_4 in grammes per litre	1·52	2·69	2·90	5·66	6·60	7·40	9·60	7·0?
Average temperature	34°	41°	35°	38°	40°	43°	40°	45°?
Weight of bacteria	0·24	0·49	0·33	0·42	0·81	0·29	0·37	0·66
Sugar employed	18·6	15·5	20·69	15·2	15·0	19·09	15·09	16·46
Sugar fermented	14·6	12·9	10·49	15·2	15·0	13·70	15·09	16·13
Duration in hours of the experiment	47	47	43	48	66	48	67	45
Weight of fat obtained	0·41	Nil	—	—	—	—	0·20	2·53

Note.—Actually the temperature in Experiments 3, 4, 7, 8 and 10 was made to fluctuate in a special manner under the belief that the variation of temperature which occurred accidentally in Experiment 3 was responsible for the synthesis of the fat which was found produced in that experiment. As has been noted, the writer was unable to repeat the phenomenon, but still more because of the results recorded in the next communication it becomes unnecessary to record the exact details as to the fluctuations of temperature to which the fermentations were submitted.

clearly represents a continuation of the process by which the lactic acid-forming enzyme comes to predominate in its action.

FIG 2



The following facts may be deduced from the experimental results recorded in Table I and the accompanying graphical representations :—

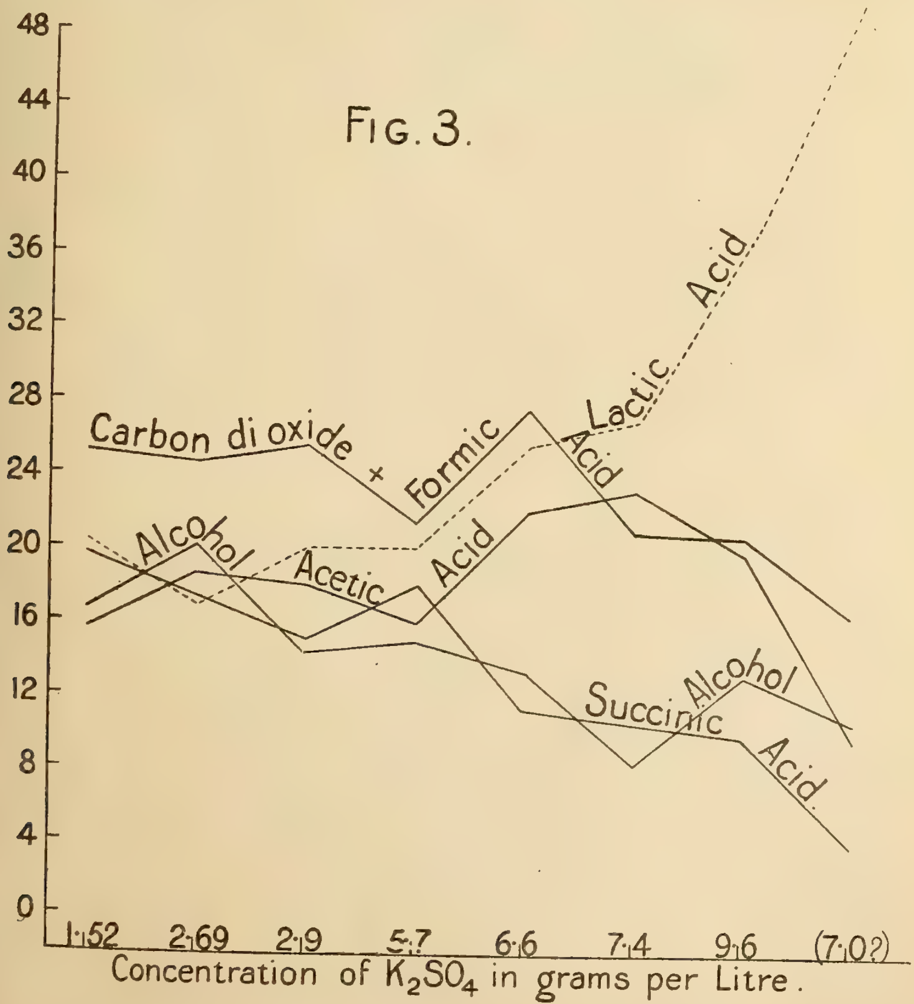
(1) The fermentation takes place in two main directions. On the one hand, there is formation of lactic acid, and, on the other, a group of substances which seem to be related more closely to one another as regards their origin than they are related to lactic acid. This group comprises alcohol, formic acid, and carbon dioxide (which arises from it by further decomposition), acetic acid and succinic acid.

(2) Succinic acid and acetic acid are complementary as regards the extent

of their formation, and are therefore probably produced from a common parent substance.

(3) The average drawn between succinic acid and acetic acid corresponds very closely with the average between acetic acid and alcohol, from which it would appear that the three substances are related to a common intermediate substance, and are produced by the same enzyme action.

(4) The curves for the production of alcohol and succinic acid closely follow one another, indicating that the oxidation of a part of the sugar to succinic acid, and the reduction of a part of the sugar to alcohol, are complementary processes.

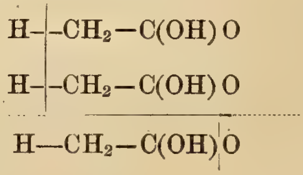


It is of interest to add together the acetic acid and succinic acid produced in each experiment. It will be seen that, in each case, the combined weight does not differ very greatly from 33 per cent. The same applies to the sum of the weight of acetic acid and alcohol. When the fermentation has taken place under certain conditions, as in Experiment 3, the sum of either of these two pairs of products no longer approximates to a constant figure, but the point to which the writer would draw attention is that, throughout conditions which have varied sufficiently to introduce considerable variation in the individual products, the sum of these pairs approaches very closely to a constant. Thus, a variation, as seen between Experiments 4 and 5, represents nearly 100 per cent. on the amount of succinic acid, and yet only about 5 per cent. on the sum of succinic acid and acetic acid. Similarly, the alcohol, as between Experiments 4 and 5, has varied by 100 per cent., but the sum of alcohol and acetic acid has only varied by 4.

	4.	5.	6.	7.	8.	10.	11.	Mean.
Acetic acid.........	15·6	23·2	16·0	18·6	18·0	22·0	19·9	
Succinic acid......	19·6	10·7	18·0	17·4	15·1	11·2	9·8	
Sum	35·2	33·9	34·0	36·0	33·1	33·2	29·7	33·6
Acetic acid.........	15·6	23·2	16·0	18·6	18·0	22·0	19·9	
Alcohol	16·6	8·3	15·0	20·0	14·3	13·4	13·2	
Sum	32·2	31·5	31·0	38·6	32·3	35·4	33·1	33·4

Doubtless, these regularities are in part due to some constancy in the conditions which is not apparent, but, nevertheless, the results are highly suggestive that succinic acid, which is, in point of fact, di-acetic acid, arises in this fermentation from the same intermediate substance which gives rise to acetic acid. And, again, since acetic acid is shown to be similarly related to alcohol as regards its origin, it seems clear that the three substances arise through the agency of the same enzyme or enzymes. These results suggest, therefore, that succinic acid may arise by the union of two $—CH_2COOH$ groups.

The close relationship of these three substances is pictorially represented below.



$$\begin{array}{l} H-|-CH_2-C(OH)\,O \\ H-|-CH_2-C(OH)\,O \\ H—CH_2-C(OH)|O \end{array}$$

Each line is equivalent to a molecule of acetic acid, and the three are thus equivalent to a molecule of glucose.

Above and to the right is succinic acid, below and to the left acetaldehyde. Ignoring the dotted line, to the left is alcohol. On the right below, the removal of oxygen by an acceptor is represented; if this acceptor is acetaldehyde the product becomes acetic acid. Above and to the left hydrogen is represented as being separated from the glucose molecule in proportion corresponding to the oxygen, the two phenomena representing the action of the reductase, the action of which it was suggested was impaired under certain conditions (Grey, 1914). The acetaldehyde is thus represented as having three possibilities as regards transformation. It may, as has been previously suggested to account for the tendency under certain conditions for alcohol and acetic acid to appear in equimolecular proportions (Harden, 1901), undergo the Cannizarro condensation into equimolecular proportions of the acid and the alcohol. It may become oxidised to the acid by the oxygen represented on the right or reduced by the hydrogen represented on the left of the scheme.

The key for the working of such a mechanism is the existence of an enzyme, or a co-operation between enzymes, capable of effecting simultaneously oxidation and reduction. Any interference with such a mechanism would lead to a simultaneous diminution in the production of alcohol, acetic acid and succinic acid. This simultaneous diminution has been established by experiment as actually occurring.

The Comparison between the Fermentation of Glucose and that of Mannitol.

In an earlier communication* the writer expressed the view that the fermentation of mannitol and glucose by *B. coli communis* was brought about by the same set of enzymes and that in general it was highly probable that bacteria dealt with all carbohydrate molecules, and molecules of substances allied to them such as the corresponding alcohols, upon a plan which was characteristic for the bacterium; in other words, that the products obtained by the action of a bacterium depended upon the bacterial content of enzymes rather than on the nature of the sugar. Only in the case of the formation of the first intermediate product might a special enzyme be necessary for a special carbohydrate configuration, but once the first intermediate product is formed the remainder of the fermentation is effected in all cases in the same way, modified only by the secondary reactions which may occur in the direction of further reduction or oxidation, should the conditions give opportunity for such changes.

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* Grey, E. C., 'Roy. Soc. Proc.,' B, vol. 87, p. 472 (1914).

It was suggested in the earlier communication that mannitol was thus in a position to give rise to a greater proportion of alcohol than glucose, not simply because it was a compound already more reduced than glucose, but because the hydrogen atoms existing in excess in the case of mannitol rendered less necessary the action of the reductase. If acetaldehyde were the intermediate substance immediately preceding alcohol, it was suggested that an interference with the reducing mechanism or reductase might lead to an accumulation of the aldehyde, which in its turn would react upon the intermediate substance A. If intermediate substance A had no alternative but ultimately to become acetaldehyde and formic acid, the accumulation of the aldehyde resulting from a weakening of the reductase would only lead to a slowing down of the fermentation as a whole, but since the intermediate substance can, according to the hypothesis, become changed by a mere molecular rearrangement into its isomer lactic acid, the result of a weakening of the reductase is a proportional increase in the production of lactic acid.

This hypothesis concords entirely with the results of experiment. Further, it would be expected to follow from this hypothesis that the more completely the phenomenon of bacterial growth and consequent change in the enzymes was excluded from the fermentation, and the less possibility there was for the products of fermentation to accumulate and hinder the reaction, both of which possibilities are minimised by the new technique, the more completely should the fermentation products from mannitol resemble quantitatively those from glucose, so that finally the only difference should be that part of the acetic acid in the case of the glucose should be represented by alcohol in the case of mannitol. How true this is may be seen by the following comparison between one of the analyses of the products from glucose already described and an analysis of the products of a mannitol fermentation carried out under the same conditions.

	Glucose.		Mannitol.	
Hydrogen	0·21		0·54	
Carbon dioxide and formic acid	27·53		27·61	
Lactic acid	25·46		23·11	
Succinic acid	11·23		12·03	
Acetic acid	22·02	35·38	10·51	37·73
Ethyl alcohol	13·36		27·22	
Total	99·81		101·02	

These results speak very strongly, if not conclusively, in favour of the writer's hypothesis that the two substances are fermented by the same set of enzymes.

With regard to the removal of the hypothetically produced acetaldehyde from the sphere of the fermentation by reduction, it may be noted that one would expect in the case of a mannitol fermentation not to be able to detect the presence of as much acetaldehyde in the solution as in the case of a similar fermentation with glucose. The writer has sought for acetaldehyde in the case of mannitol fermentation and has failed to detect it, a fact in harmony with the above consideration.

Summary and Conclusions.

By allowing *B. coli communis* (suspended in saline solution) to act on glucose it has been found that the proportion between the products of decomposition differs considerably from that obtained in the earlier experiments in which the organism was allowed to grow in a mixture of glucose and peptone, a greater proportion of alcohol, acetic acid, and succinic acid, and smaller proportion of lactic acid being obtained.

The conclusions which may be drawn from the results refer in the first place to *B. coli communis* in particular, and in the second place to bacterial fermentation in general.

In particular it has been shown that:

(1) Succinic acid has an origin in common with acetic acid and alcohol.

(2) The formation of lactic acid is independent of the formation of the above three products.

(3) The enzymes which effect the decomposition of glucose also co-operate in the decomposition of mannitol.

With regard to bacterial fermentation in general the experimental results point to the independence of the intracellular ferments.

The experiments of Harden and Penfold,* and later of the writer,† upon *B. coli communis* grown in the presence of a chloroacetate showed that by artificial selection a strain could be obtained which yielded products from glucose in different proportions from those in which they were formed by the original strain, and since this difference in proportion of products must correspond to a difference in proportion of the enzymes forming them, or of the activity of these enzymes, which for practical purposes is the same thing, it is clear that artificial selection can vary the proportion between certain enzymes, and this is good evidence that they are independent of one another in the original cell. The further results here recorded give additional evidence of this, for they show that with the unselected organism, even when the whole process of fermentation only occupies 48 hours, considerable variation in the proportion of the products may be obtained by varying the conditions.

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* Harden, A., and Penfold, W. J., 'Roy. Soc. Proc.,' B, vol. 85, p. 415 (1912).

† Grey, E. C., 'Roy. Soc. Proc.,' B, vol. 87, p. 472 (1914).

With regard to the results of exactly comparable experiments with mannitol, good evidence has been obtained in confirmation of the view already put forward by the writer that the fermentation of various carbohydrates and allied substances by bacteria is brought about by a single set of enzymes whose actions are common to all such cases of fermentation. This does not exclude the possibility that the first step in the degradation of a particular molecular structure may require a special enzyme in order to produce the first intermediate substance, which according to the writer's hypothesis would be the same for all analogous cases of fermentation.

The Enzymes Concerned in the Decomposition of Glucose and Mannitol by Bacillus coli communis. Part III.—*Various Phases in the Decomposition of Glucose by an Emulsion of the Organisms.*

By Egerton Charles Grey (Beit Memorial Research Fellow).

(Communicated by Dr. A. Harden, F.R.S. Received July 25, 1917.)

(From the Laboratory of Prof. A. Fernbach, Institut Pasteur, Paris.)

CONTENTS.

	PAGE
Apparatus for the Study of the Various Phases of a Fermentation	93
Details of an Experiment in which the Products of Fermentation have been Examined at Successive Stages	95
General Considerations	102
Summary and General Conclusions	104

In Part II of this series results have been described which indicate the existence of several independent processes occurring during the one experiment. The experiment which will be now described was undertaken with the object of simplifying results by shortening the time of the fermentation. Arrangements were made also to count the bacteria at various periods, with the object of determining how far the fermentation was due to enzyme action which could be said to be carried on independent of the multiplication of the cells. The plan of the experiment was as follows:—About 50 grm. of glucose was to be fermented in a volume of 5 litres of solution. A sample of about a litre was to be removed every 12 hours and submitted to analysis. By a comparison of these analyses the amounts of the various products which had been formed during each interval of time would be determined.

Certain modifications were necessary in the form of apparatus used and in regard to the analysis. With regard to the analysis, the estimation of carbon dioxide from time to time during the course of the experiment required a special device. In the first place, the gas space above the solution would gradually increase as the samples of solution were removed, and diffusion from the solution to the space above would cause the concentration of CO_2 in the solution to be constantly changing, and in the second place, since the sample of the fermentation solution would have to be removed while warm, precautions would have to be taken to avoid loss of CO_2 prior to its mixture with standard barium hydroxide solution. The apparatus was therefore arranged according to the plan represented in the figure below.

Arrangement of Apparatus for the Study of the Various Phases of a Fermentation.

The essential differences between the apparatus for the study of the fermentation in stages, and that (fig. 1 of Part II) for the case of a complete fermentation are—the flask A for the withdrawal of the samples of the solution ; the gas burette H for the removal of samples of gas and estimation of the carbon dioxide ; and the arrangement of the three-way tap K by means of which the system of bottles, L′, L″, L‴, containing the alkali for the absorption of the carbon dioxide evolved during the fermentation, may be shut off from connection with the fermentation flask D, while the hydrogen which has collected in the reservoir M is returned to the fermentation flask to replace the volume of the liquid removed at A.

The method of operating is as follows:—The flask D at the beginning of the experiment contains about 5 litres of solution, and the air space above the liquid is about half a litre. The pinchcock C is closed, the taps H and K are closed, and F and G are opened. By the aspiration of the water-pump with which G is in connection, the flask D is deprived of air, the solution being brought to the boil under the reduced pressure. Nitrogen is introduced through the tube B after removal of the flask A. During the course of the fermentation the three-way tap K is turned so that the evolved gases pass through the bottles, L^1, L^2, L^3, into which a known amount of CO_2-free alkali has been introduced. The bottles are arranged so that they may be readily removed, and the amount of carbon dioxide absorbed determined.

In order to ascertain the amount of carbon dioxide which has been produced up to any moment of the fermentation, it is necessary to estimate the gas which has been absorbed by the alkali in the bottles, L′, L″, L‴, the amount which is present in the fermentation solution, and the amount which is in the gas space in the fermentation flask. These two last must be determined simultaneously owing to the possibility of repartition of the carbon dioxide between the two media by diffusion. The arrangement of the apparatus permits of this simultaneous determination. The taps F and K are closed, and H and G opened; mercury is thus drawn up into the burette; G is then closed. A sample of the solution in the flask is removed for the estimation of dissolved carbon dioxide by connecting an evacuated flask containing a quantity of standard barium hydroxide solution with the tube B and opening the pinchcock C, as described in the section on the estimation of carbon dioxide. At the instant that the sample of solution is removed, the tap F is opened, and a sample of gas thus drawn into the gas burette by the fall of the mercury. A small piece of solid potassium hydroxide is allowed to float up into the burette for the absorption of the carbon dioxide in the sample of gas.

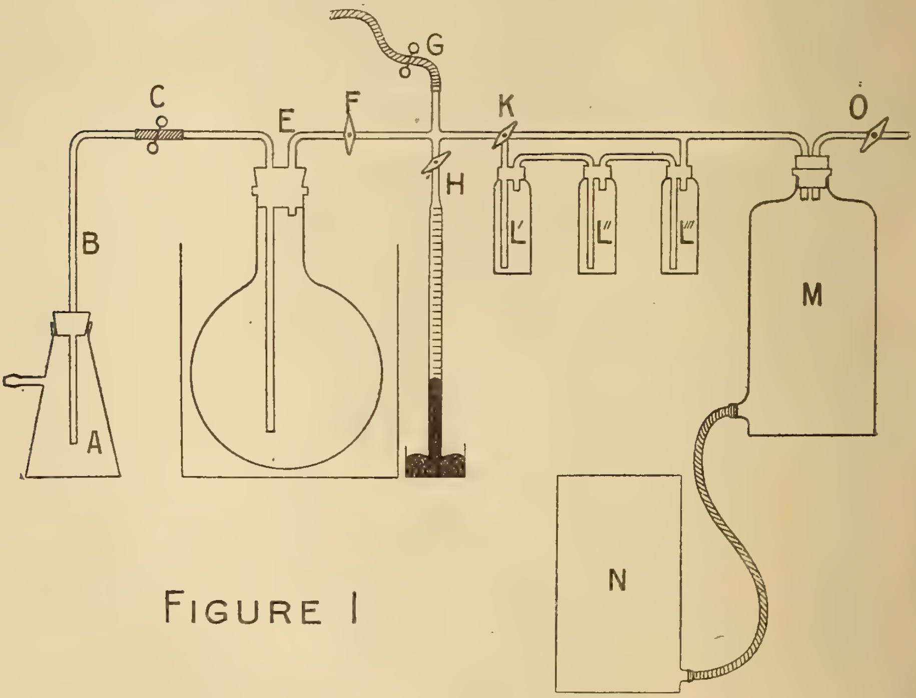


FIGURE I

Immediately after the sample of solution has been drawn off into the evacuated flask for the determination of the carbon dioxide dissolved, a much larger sample is withdrawn into the flask A, which is slipped over the tube B, which already supports a rubber stopper. To withdraw the sample the flask A is evacuated, and the tap K is turned so as to shut off the absorption bottles and put the fermentation flask into direct communication with the gas-holder M. The vessel N is raised. Now upon opening the cock at C the sample is drawn into A and the hydrogen and nitrogen in M are returned to the fermentation flask.

The hydrogen can be estimated either by the measurement of the change in volume of the gas or by direct determination. At the end of the experiment, when all the samples of solution have been removed from the fermentation flask, the gases may be displaced by the introduction of hot water through B, and collected in M.

The arrangement of apparatus is suitable for following the stages of either aërobic or anaërobic fermentations.

Details of an Experiment in which the Products of Fermentation have been Examined at Successive Stages.

The experiment was carried out as follows:—A weight of 45·84 grm. of pure anhydrous glucose was dissolved in distilled water and the solution sterilised. This solution was then added to about 3 litres of sterile distilled water in a flask of 5½ litres capacity, in which also was about 40 grm. of chalk contained in small floating sacks. The sugar was thus not sterilised in contact with the chalk. The contents of the flask were cooled to 37° C., and 750 c.c. of an emulsion of bacteria was added. The emulsion of bacteria was made from the surface of agar by means of a solution of potassium and magnesium sulphates. The amount of each introduced finally into the solution to be fermented was 11·6 grm. of potassium sulphate and 3·3 grm. of magnesium sulphate. The total volume of the solution in the flask at the beginning of the experiment was 5100 c.c. The fermentation was allowed to take place at approximately 40° C. The weight of bacteria introduced corresponded to a dry weight of 0·61 grm., and the weight of other organic matter washed from the agar bottles was 1·83 grm.

The number of living bacteria introduced at the beginning of the experiment was $5100 \times 217 \times 10^{12}$ or 217×10^{12} for each cubic centimetre of the solution in the flask.

At the end of 12 hours from the time of introduction of the bacteria samples of the solution were removed for the estimation of the various products resulting from the fermentation and also for the enumeration of the living bacteria. The operation was repeated at 24, 48 and 72 hours from the commencement of the experiment. The number of bacteria living was determined in each case by plating out on nutrient agar.

The experiment was thus divided up into four periods. The nature of the change during any period was ascertained by a comparison between the composition of the fermentation solution at the beginning and end of the period. The changes in the number of the bacteria and the extent to which the sugar was fermented during each period may be seen from the following figures.

Period.	Duration.	Number of bacilli per c.c. of the solution at the beginning of each period in milliards.	Amount of sugar at the start of the period.	Amount of sugar at the end of the period.	Amount of sugar changed during the period.	Volume of the solution during fermentation.
	hrs.		grm.	grm.	grm.	c.c.
1	12	217,000	45·84	35·06	10·78	5100
2	12	4·5	30·95	20·41	10·54	4400
3	24	1,400,000	16·79	10·09	6·70	3620
4	24	450,000	6·84	3·3	3·54	2214
	72					

The following facts may be noted. During the first period of 12 hours there is an enormous falling off in the number of living bacteria, and during the second period of 12 hours there is a still greater increase in the number which are living. Subsequently there is a diminution also but proportionally small as compared to the diminution during the first period. In spite of the fact that the first period is characterised by rapid diminution and the second period by rapid multiplication of the bacteria, nevertheless the amount of sugar transformed into products which do not reduce copper solution is approximately the same in both cases.

The sample which was removed for analysis at the end of the first 12 hours gave the following results. In the result must also be included the carbon dioxide evolved during this period.

Result of Analysis of the Fermentation Solution at the end of the First Period of 12 Hours.

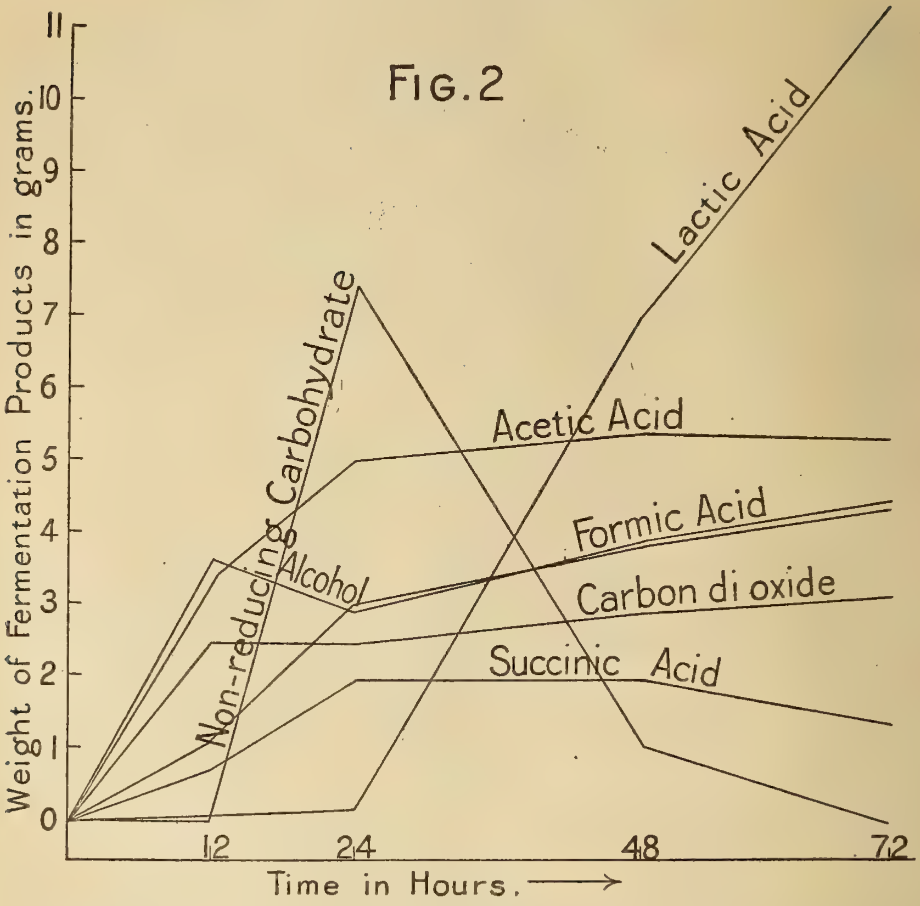
	grm.	grm.
Weight of sugar fermented		10·78
Products found—		
Carbon dioxide	2·43	
Formic acid	1·09	
Acetic acid	3·40	
Succinic acid	0·72	
Lactic acid	0·10	
Ethyl alcohol	3·56	
Hydrogen, 420 c.c. at N.T.P	0·04	
	——	11·34
Ratio CO_2/H_2 .... 1·4		

This result is of considerable importance, for it shows that during the period of death of the bacteria the production of lactic acid ceases. The fact that ethyl alcohol, acetic acid and (formic acid + carbon dioxide), each represent about a third of the sugar fermented, and that the proportion between these substances is the same as when the fermentation is carried out under very different conditions, speak strongly in favour of the view already put forward as to the origin of these substances in common from a single intermediate substance, and by the action of the same enzyme. The fermentation during the first period has in fact occurred in the manner which was to be expected from the experiments described in Part II provided it were possible to exclude the action of the lactic-acid-producing enzyme, a result which has here been achieved. It has been shown in Part II that succinic acid has an origin probably in common with acetic acid and that the two substances could replace one another; it requires therefore no change in the writer's hypothesis to account for the variations in the amount of succinic acid produced in these experiments.

Here, as in the earlier experiments of Harden and later also of the writer, the tendency is seen for the alcohol and acetic acid to be produced in equimolecular proportions.

In order to ascertain the proportion of the products formed during the subsequent periods of the fermentation, it is necessary to subtract from the amounts of the products found in each period the amounts of such substances which were present at the commencement of the period under consideration. These results may be deduced from a study of fig. 2, which is a graphical record of the rate of formation of the various products of the fermentation.

FIG. 2



Products of the Fermentation of Glucose formed during the Second Period of 12 Hours.

	grm.	grm.
Weight of sugar fermented		10·54
Products found—		
Carbon dioxide	0·028	
Formic acid	1·895	
Acetic acid	1·620	
Succinic acid	1·158	
Lactic acid	0·16	
Ethyl alcohol	Nil.	
Non-reducing carbohydrate*	7·40	
		12·26
Hydrogen	about 50 c.c.	

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* Calculated on the assumption that the reducing sugar formed on hydrolysis has the same reducing power as glucose.

Since the products add up to a greater weight than can correspond to the sugar fermented, it is possible that the weight of the carbohydrate synthetised by the bacteria cannot be quite accurately calculated from the reduction of copper solution after hydrolysis. It is also possible that the additional weight is derived from the organic matter introduced with the bacteria or formed during the autolysis of the bacteria in the first period.

But the conclusions which may be drawn from the analysis recorded above are not affected by the fact that the products obtained are somewhat in excess of the sugar used. The quantity of sugar which has been transformed or synthesised into a form in which it does not reduce copper solution until after hydrolysis represents about 70 per cent. of the original glucose.

The great importance of this result will be considered later. For the moment it will suffice to note the following facts with regard to this period of the fermentation.

It is essentially a period of synthesis. There is practically no production either of alcohol or carbon dioxide or hydrogen. There is, as in the first period, no formation of lactic acid. On the other hand, there is here a formation of succinic acid in greater proportion to the acetic acid than in the first period.

The products formed during the third period, *i.e.* during the second 24 hours of the fermentation, were determined, as in the case of the second period, by difference.

The results were as follows:—

Products of the Fermentation of Glucose formed during the Third Period (24 *to* 48 *Hours*).

	grm.	grm.
Weight of glucose fermented	6·70	
Weight of non reducing carbohydrate fermented	4·98	
	——	11·68
Products found—		
Carbon dioxide	0·34	
Formic acid	0·82	
Acetic acid	0·46	
Succinic acid	0·02	
Lactic acid	6·63	
Ethyl alcohol	1·05	
	——	9·32

Hydrogen not estimated.

It will be seen that there is a loss here which almost corresponds in magnitude to the gain of the previous experiment and that the two results taken together would balance. This seems to indicate that the error lies in the determination of the residual glucose by the reduction of copper.

The most striking fact observed in the result of the third period of fermentation is that it is mainly a lactic acid production; 60 per cent. of the carbohydrate fermented has been transformed into lactic acid.

The synthetic period during which there is practically no lactic acid formed is immediately followed by a degradation process both of the sugar in the solution and also the carbohydrate previously elaborated. This degradation period is characterised by the formation of a very high yield of lactic acid, which in part, at least, comes from the carbohydrate previously synthesised. The production of alcohol, which had ceased during the period of rapid growth and multiplication of the bacteria, viz., the period of synthesis, commences again in the ensuing period of degradation. The second period of alcohol production, like the first, is a period of death for the bacteria.

Products of the Fermentation of Glucose formed during the Third 24 *Hours of the Fermentation.*

	grm.	grm.
Weight of glucose fermented	3·41	
Weight of non-reducing carbohydrate fermented	0·67	
	——	4·08
Products found—		
Carbon dioxide	0·37	
Formic acid	0·62	
Acetic acid	Nil.	
Succinic acid	Nil.	
Lactic acid	2·86	
Ethyl alcohol	0·39	
	——	4·24

Hydrogen not estimated.

It will be seen that the fermentation that has taken place during this interval is in the main the same as during the preceding interval of 24 hours. The whole period is chiefly characterised by the production of lactic acid.

The excess of formic acid over alcohol is not in keeping with the results of previous analyses, for it has always been found that the ratio of alcohol and acetic acid to formic acid is a constant,* but there are reasons to doubt the correctness of this formic acid figure obtained here, since it was found that the solution of sodium formate gave a precipitate of iodoform in the cold on the addition of sodium hydroxide and iodine, and it is possible that the substance causing this reaction may also have reduced mercuric chloride. It was observed that the precipitate of mercurous chloride obtained in this experiment in the estimation of formic acid was blackened. The figures for this fourth analysis are derived by the consecutive subtractions from the previous analyses, and it would therefore not be safe to insist too much upon small differences here, especially as the total amount of sugar fermented during this last interval is small.

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* Grey, 1914.

It is against the principles indicated in the commencement of this communication to prolong the period of a fermentation beyond the time during which the main primary changes occur, and in the discussion of these results therefore conclusions will alone be considered in as far as such can be supported by the evidence of the first 48 hours. Even this period is far too long and it will be the object of future researches to reduce it.

The balance sheet of this experiment is made out in the following Table.

Table II.—Balance Sheet for the Experiment on the Phases of the Decomposition of Glucose by *B. coli communis.*

Time from the beginning.	Carbohydrate decomposed.		Products recovered.	
	Glucose.	Non-reducing carbohydrate.	Of degradation.	Of synthesis.
hours.				
12	10·78	—	11·30	—
24	10·54	—	4·86	7·40
48	6·70	4·98	9·32	—
72	3·41	0·67	4·08	—
	31·43	5·65	29·56	7·40
	37·08		36·96	

For the sake of easier comparison the results are grouped together in Table III, the products being represented as percentage of the total weight of products of degradation in each period. The balance sheet shows that there is no total loss during these four parts of the experiment, but, owing to the difficulty of being certain as to the amount of glucose as determined by reduction of copper solution, it may be that the proportion between reducing and non-reducing carbohydrate during the intermediate periods is not of the same order of accuracy as the determination of the ultimate degradation products. Since, however, the non-reducing substance produced during the second 12 hours is subsequently degraded, it does not in the end change the results.

Table III.—The Results of the various Periods of the Fermentation expressed as Percentages of the Amount of Total Products obtained in each Period, ignoring the Temporary Formation of Non-reducing Carbohydrate.

	1.	2.	3.	4.
Carbon dioxide	21·50	0·50	3·70	8·70
Formic acid	9·61	39·00	8·80	14·60
Alcohol	31·50	Nil.	11·30	9·20
Acetic acid	30·10	33·40	4·90	Nil.
Succinic acid	6·37	23·80	0·20	Nil.
Lactic acid	0·88	3·30	71·10	67·50

The results have been calculated to the amount of total products obtained in the period rather than upon the weight of sugar fermented during the period owing to the presence of the non-reducing carbohydrate, *i.e.*, carbohydrate which does not reduce copper solution until after hydrolysis. As has been pointed out, it is not possible to be sure of the reduction figure. Moreover, the object of the above Table is to bring out the comparison between the degradation processes occurring during each period, and this comparison is only obscured by the calculation of the results to the sugar which has disappeared, since part of the sugar becomes synthesised into the non-reducing carbohydrate which later undergoes degradation.

General Considerations.

Before discussing the broad conclusions which may be drawn from these researches, the writer would put forward certain considerations with regard to the methods which these results, and the results of previous work also, suggest should be adopted in future work on bacterial fermentation. These suggestions are put forward partly because this branch of chemistry is essentially one in which the co-operation of several workers is needed, and also because the results obtained by adopting the method of research described in this communication warrant the conclusion that a more extended application of these methods would help to elucidate the problems of bacterial enzyme action. With comparatively few experiments it has been possible to show that the enzymes of *B. coli communis* are, partly at least, independent of one another in their action; that the degradation of glucose is brought about by means of these independent enzyme fermentations acting either simultaneously or consecutively, and that the same set of enzymes produced by the bacterium serve for the degradation of substances allied to glucose, such as mannitol. It has been shown also that the nature of the fermentation products, and the proportion in which these appear in the final analysis, will depend on the extent to which the various enzyme actions co-operate, which in turn depends on conditions such as concentration of salts and temperature. It has been shown also that the synthetic side of the process of bacterial fermentation is quantitatively of the same order as the decompositions which follow.

Some of these facts have so far been hidden because the various changes occur rapidly, and previous experiments have been too prolonged to serve for their investigation. In the light of these results it is of interest to consider what the objections are to prolonged fermentations if such experiments aim at a characterisation of the enzymes of any particular organism at any particular time. For it is important to insist that a organism is not constant as regards the enzymes it contains, but that its composition in this respect will depend upon its immediate past history, and thus only a superficial idea of the fermentation processes set up by the organism is given by a study of the action as a whole, and it is necessary, in order that any true uniformity should be obtained, that the actions of the separate enzymes should be studied.

The objections are as follows:—

(1) The strain of the organism introduced at the start may vary with the production of new enzymes.

(2) Even if the enzymes remain constant in kind and amount their actions may be selectively impeded by the conditions of the experiment at the start, or by the accumulation of the products of the fermentation.

(3) Even if the enzyme actions were unimpeded by the conditions imposed, the prolongation of the experiment would give nothing but the sum of the various actions concerned.

For, if an organism be introduced in a small seeding into an artificial medium, the enzymes which are developed in the subsequent generations of the organism may not necessarily be the same as those which were present at the start. There may be adaptation to the conditions in the sense of the production of enzymes which bring about the decomposition of the substance under investigation along simpler lines. As an example of such a selection taking place during a single fermentation experiment, it will suffice to refer to an observation made by the writer in an earlier communication, namely, that after the fermentation of mannitol by certain strains of *B. coli communis* had come to a standstill as regards the production of gas, the strain which could now be isolated from the solution differed from the original strain in that it now no longer produced gas from mannitol even when inoculated into a fresh solution.

Again, although the strain introduced at the beginning of the experiment should remain constant during subsequent generations, as regards the production of enzymes, yet the action of these enzymes would be modified by the progressive change in the conditions resulting from the accumulation of the products of their action. If such products affected equally all the enzymes, then the result might simply be a slowing down of the fermentation process as a whole, but if, as is more likely, the enzymes are differently influenced, it may be that the course of the fermentation would vary from time to time if a prolonged fermentation experiment were made. Grimbert has shown this to be the case with a bacillus producing butyric acid, and Fernbach and Schoen have shown that the course of yeast fermentation can be diverted into channels wherein there is a large production of acids, especially interesting amongst which is pyruvic acid.

It may perhaps be pertinent to remark in this connection that in all experiments in which chalk or other alkali is added to the medium there is probably a tendency to the encouragement of the action, if not of the production, of acid-forming enzymes.

Moreover, even if the enzymes introduced at the start remained constant throughout the experiment and their actions were not selectively impeded by the products of the fermentation or by other conditions, the experiment would not serve to distinguish the products of one enzyme action from those of another. To do this it is necessary to know whether the various enzyme actions occur simultaneously or consecutively. In the first case, in order to study one of the actions, it may be helpful to be able to arrange the conditions so that the other reactions are brought to a standstill, and, in the second case, it may be that by reducing the period during which the fermentation is studied, it is possible to separate the various phases from one another. Both of these methods have been successful in the experiment described in this communication. During the first period the conditions of the experiment were accidentally such that the lactic acid enzyme did not act, and it was possible therefore to observe which substances were produced independent of it, and during the second 12 hours of the experiment it was possible to observe the importance of the synthetic side of the fermentation process, this period being really the period of rapid growth of the organism.

The method of research here described indicates the means by which the subject of the enzymes of bacteria can be more closely studied than has been hitherto possible.

Summary and General Conclusions.

By analysis of the products resulting from the decomposition of glucose by *B. coli communis*, it has been shown that the fermentation is made up of several phases which can be separately studied if the period during which the experiment is made is sufficiently short. These phases are largely to be correlated with increase and diminution in the number of living cells which are present at any time.

It has been shown that during the period which was characterised by rapid death of the cells, there was no formation of lactic acid, whereas during a period which immediately followed the rapid multiplication of the cells, lactic acid was produced to the extent of 70 per cent. of the sugar consumed. The period in which the cells died was characterised by the transformation of sugar into alcohol, and formic, acetic, and succinic acids.

Also it has been shown that at this period sugar is decomposed to a greater extent than during the period of extensive multiplication, which points to the conclusion that the fermentation is brought about by enzymes.

During the period of multiplication there is a transformation of glucose into a more complex substance which only reduces copper solution after hydrolysis. The extent of the synthesis during this period is quantitatively of the same order as the degradation which is brought about in the period which follows.

From these results the following general conclusions may be drawn:—

Elaboration of glucose into more complex material may occur during certain phases of the life of bacteria to an extent exceeding the degradation brought about during the same time, so that it were a fallacy to suppose that bacteria are as a class concerned chiefly with the business of decomposition. The degradations which these organisms set up are the sequel of those processes of synthesis which are more immediately associated with the growth and multiplication of the cells. In 48 hours a synthesis of 3 grm. of fat has been observed when less than a gramme of bacteria was mixed with glucose under conditions unfavourable for the normal fermentation process, and during a period of 12 hours accompanied by rapid cell multiplication, a transformation of 7 grm. of glucose into a more complex carbohydrate has been obtained. Thus it will be seen that the synthetic side of the fermentation process brought about by bacteria has been hidden because of the rapidity with which the processes of degradation supervene. In order to study the process of synthesis the fermentation must be interrupted as a rule before 24 hours have elapsed.

The second general observation which may be made is with regard to the existence of enzymes in bacteria. No one has doubted, since Buchner's demonstration of the existence of zymase in yeast and of certain other enzymes in bacteria, but that the fermentation processes brought about by bacteria and other organisms were the result of enzyme action.

Nevertheless, the independent existence within the cell of enzymes (if by definition such enzymes act independently of the life of the cell) has been, in the case of bacteria at least, rather a matter of speculation.

It may be suggested here, however, that it is not necessary to isolate from cells an unorganised material capable of bringing about a fermentation in order to demonstrate that such a fermentation is brought about by enzymes (unless such substances were defined as enzymes only provided they could be isolated by the present means at our disposal). If an enzyme is regarded as a substance capable of inducing fermentation independently of the life of the cell, then there are two methods of demonstration which serve to establish the existence of enzymes in any particular case without the necessity of separating them from the cell. The first method consists in carrying out the fermentation under conditions which do not support the life of the organism. The first part of the experiment described in this communication very closely approaches such conditions. Here it was shown that the amount of sugar decomposed during the rapid diminution in the number of living cells was as great as during that period where the number of living cells was both at the start and the finish enormously greater.

The second method, it is suggested, of demonstrating without actual destruction of the cell that the fermentation is brought about by enzymes, depends upon the proof that the several fermentation phenomena are independent of one another. For if a series of functions of a cell are absolutely independent of one another, some of them, at least, cannot be essential to the life of the cell.

Both methods here referred to have found application in the experiments described in this communication, and, although the separation of the phases of the fermentation was not absolute, either as regards complete absence of living cells at any one time, or complete transformation of glucose in one direction only, nevertheless taken in conjunction with the earlier work of Harden and Penfold, and later of the writer, the present results leave little room for doubt that the several fermentation processes by means of which *B. coli communis* brings about the decomposition of glucose and allied substances are true enzyme actions, and are capable of acting independently of one another, and thus breaking down the sugar in various ways.

This conclusion is probably applicable to other cases of fermentation.

In conclusion I would express my thanks to Prof. Auguste Fernbach for his kind hospitality and for valuable criticism.

PROCEEDINGS
OF
THE ROYAL SOCIETY.

Series B. Vol. 90. No. B 626.

BIOLOGICAL SCIENCES.

CONTENTS.

Page

The Germicidal Action of Ultra-violet Radiation and its Correlation with Selective Absorption.—Addendum. By C. H. BROWNING, M.D., and SIDNEY RUSS, D.Sc. 107

CROONIAN LECTURE:—Evolution and Symmetry in the Order of the Sea-pens. By Prof. SYDNEY J. HICKSON, F.R.S. . . . 108

Bactericidal Properties Conferred on the Blood by Intravenous Injections of Diamino-Acridine Sulphate. By C. H. BROWNING and R. GULBRANSEN 136

The Isolation and Serological Differentiation of Bacillus tetani. By W. J. TULLOCH, M.D., Temp. Capt. R.A.M.C. (Lecturer on Bacteriology at the University of St. Andrews) 145

Obituary Notices of Fellows deceased:—Hermann Graf zu Solms-Laubach (with portrait); Robert Bell . . . . xix—xxvii

PRINTED FOR THE ROYAL SOCIETY AND SOLD BY
HARRISON & SONS, 45, ST. MARTIN'S LANE, LONDON, W.C.

Price Three Shillings.

No. B 626.

April 2, 1918.

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The Germicidal Action of Ultra-violet Radiation and its Correlation with Selective Absorption.—Addendum.

By C. H. Browning, M.D., and Sidney Russ, D.Sc.

(Communicated by W. B. Hardy, Sec. R.S. Received November 6, 1917.)

Our attention has been drawn to the work of Barnard and Morgan,[*] mention of which was unfortunately omitted from our paper on "The Germicidal Action of Ultra-violet Radiation and its Correlation with Selective Absorption."[†]

Our work was suggested by the necessity for a strictly scientific analysis of recent statements regarding (1) the differentiation and isolation of allied organisms on the basis of varying susceptibility to the lethal action of this radiation; and (2) the possibility of exercising a therapeutic effect in deep-seated infective lesions by exposing the adjacent body surface to a source of the radiation.

We regret that the above-cited communication was unknown to us at the time, since the authors have investigated the problem of bactericidal action by ultra-violet radiation by a method in general similar to that which we adopted, although their results differ from ours in several respects. Thus, these authors state that, for a number of organisms, the germicidal radiation ranges between wave-lengths 3287 and 2265, but they do not refer to differences in vulnerability among the various organisms. Our own observations showed that the most germicidal region of the ultra-violet spectrum of the tungsten arc lies between wave-lengths 2940 and 2380, and that, with prolonged exposure, the extent to which bactericidal action occurs on either side of these wave-lengths depends upon the particular organism investigated. With regard to selective absorption of the rays, the authors state: "We have been unable to find that any of the radiations contained in the spectrum are penetrative to organic substances such as agar, or dead animal or vegetable tissue. Neither can they penetrate living tissues, but we are continuing experiments in this direction to more exactly determine their action." Although it appears that they had given consideration to this point, the correlation of the germicidal action with the remarkably sharp selective absorption of the lethal radiations exhibited by suspensions of bacteria, solutions of protein, etc., which we have demonstrated, does not seem to have been put to experimental test.

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[*] 'Roy. Soc. Proc.,' vol. 72, p. 126 (1903).

[†] 'Roy. Soc. Proc.,' B, vol. 90, p. 33.

CROONIAN LECTURE: *Evolution and Symmetry in the Order of the Sea-pens.*

By Prof. SYDNEY J. HICKSON, F.R.S.

(Lecture delivered June 22, 1916,—MS. received November 19, 1917.)

1. *On Radial and Bilateral Symmetry in the Animal Kingdom.*

In a general survey of the animal kingdom two kinds of body symmetry are found—the bilateral and the radial. In many cases, genera, families, and even classes of animals show some structural departures from the completely radial or bilateral symmetry; in others, there is a combination of the two symmetries, as when an outer body form of radial symmetry covers bilaterally symmetrical organs, and, further, there is now conclusive evidence that in the course of the evolution of certain groups of animals one form of body symmetry has been supplanted by the other. With all the varieties of form and structure adapted to the different conditions of life, and with all the complexities of development and organisation due to phylogeny, there are but few examples of animals that are completely bilateral or completely radial as regards all their organs, but the dominance of one or other of these symmetries is, in most cases, so far pronounced that any animal can be placed in its proper group on this method of classification.

It is not possible to give in a few words a comprehensive definition of what is meant by the two symmetries; but if attention is confined for the moment to such types as the earthworm or a fish on the one hand, and to a polyp or a jelly-fish on the other hand, a basis for a definition may be found for each of the two symmetries. Thus, a bilaterally symmetrical animal is one in which the principal organs and appendages of the body are arranged in pairs on either side of a median vertical plane. Such an animal exhibits an anterior and a posterior extremity, a dorsal and a ventral surface, and a right and left side. And a radially symmetrical animal is one in which the principal organs and appendages of the body are arranged symmetrically on radial lines or planes proceeding from a common centre or a common axis. Radially symmetrical animals may be spherical or oval, dome- or disc-shaped, or cylindrical in form, and they do not exhibit anterior and posterior extremities, dorsal and ventral surfaces, nor right and left sides.

Animals that are bilaterally symmetrical are usually free, and propel their bodies actively through the medium in which they live by powerful muscular movements of the body wall or of the appendages. Animals that are radially symmetrical, on the other hand, are usually either sedentary in habit, or have a floating or drifting habit with the aquatic plankton.

There is clearly an intimate association between the symmetry and the habit. An animal that is active and moves rapidly from place to place in search of food or shelter must be bilaterally symmetrical. The animal has been constructed by the forces of Nature in the same way as man constructs his railway trains, steamships, and aeroplanes, with a view to rapidity of progress combined with economy of power.

An animal that is sedentary or drifting in habit, one that does not use its muscles for driving its body through the water, through the air, or on the surface of the earth, does not need this exquisite balance of parts on a median vertical plane. It may be any shape that is not inconsistent with the conditions of the environment, as we see in variations of shape and ramification of corals and sponges. But, provided that the forces that act upon it and the food upon which it preys come to it with equal intensity from all directions, it has a tendency to assume a more or less perfect radial symmetry.

We can see the influence that habit has had in determining the symmetry of the body in the evidence of phylogeny afforded by the embryonic development of several kinds of animals.

Thus, the Crustacea are, as a rule, animals with perfectly bilateral symmetry of the body, and they are bilaterally symmetrical from the early embryonic stages until adult life is reached, and all the time they are active and free. But in the order Cirripedia we find a series of forms in which the bilateral symmetry of the external shell is gradually obscured until we reach the almost perfect radial symmetry of such a genus as Coronula. The nauplius and the cypris larval stages of these Cirripedes are free and, like other Crustacea, bilaterally symmetrical, and it is only in the adult and sedentary stage that the tendency to the assumption of a secondary radial symmetry shows itself.

Again, in the Echinodermata we find a dominant radial symmetry, associated in many of the Crinoidea with a sedentary habit, and in the forms that are not strictly sedentary the movements are slow and indeterminate in direction. The embryological evidence that the Echinoderms have passed through, in their phylogeny, firstly a free bilaterally symmetrical stage is almost conclusive; but it is even of greater interest to find that in some of the Holothuroidea, a group in which the movements in a definite direction of the body are assisted by a more powerful development of muscles in the body wall, several genera—such as the Elasipoda—exhibit a very pronounced return to a bilateral symmetry of the body.

In comparing the animals that show a dominant radial symmetry and are sedentary or drifting in habit with those that show a dominant bilateral symmetry and are free and active in their movements, it may be observed that the former are far more variable in external form and in the number and arrangements of their organs than the latter.

If we take such a form as Hydra as an example of a sedentary animal, or Aurelia as an example of a drifting animal, with radial symmetry, it is found that, within the limits of what is at present regarded as a simple species, profound variations in the important organs are of frequent occurrence. In Hydra the number of tentacles and the number and form of the gonads vary within a wide range in any collection of the same species taken from the same locality. In Aurelia* and in Eucope† there are numerous variations to be found, when a large collection of specimens is examined, in the number and arrangement of the gonads, the gastric pouches, the radial canals, the sense organs, and the tentacles, and if we add to these variations in the important organs the minor variations in size, colour, length, or size of the parts, etc., which are reckoned of some importance in systematic description, the variability of these forms is found to be of great importance. If we compare the extent of these variations with the variations seen in a typical bilaterally symmetrical animal such as an earthworm or a crayfish a great difference is observed. Thousands of specimens of a species of earthworm may be examined without finding any variations in the number or position of the gonads, of the œsophageal pouches, or of the principal blood-vessels. Similarly, variations in the number or form of the appendages of a crayfish are very rarely met with, and major variations in the viscera are so rare that only a few isolated cases have been recorded.

Many other examples could be quoted to illustrate this point of difference between radially symmetrical and bilaterally symmetrical animals, but a great many instances could also be brought forward which seem to be exceptions to what we might otherwise call a biological rule.

I have frequently been impressed, in my systematic work on Cœlenterata, with instances of the rigidity of certain characters in some species or genera or even larger groups of these radially symmetrical organisms. The colour of the spicules of *Tubipora musica* is always red, there is always a blue colour in the corallum of Heliopora, the spicules of Eunicella are always torch-shaped, *Paragorgia arborea* is always dimorphic, there are always eight mesenteries in the Alcyonarian polyps and never more than eight tentacles.

These characters, which in other genera and orders are very variable indeed, seem to have become rigid or invariable in the cases quoted. No reason can, at present, be given to account for this rigidity of certain characters in a few of the many thousands of sedentary individuals and colonies, but they cannot be put forward as a controversion of the general statement that sedentary animals are usually more variable as regards their principal organs than animals that are free and active in their movements.

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* Ehrenberg, 'Abh. k. Ak. Wiss.,' Berlin, 1835.

† Agassiz and Woodworth, 'Bull. Mus. Comp. Zool.,' Cambridge, 1896.

It is not surprising that bilaterally symmetrical animals should be less variable than radially symmetrical animals. In order to secure the greatest possible efficiency of movement in any direction it is necessary that the organs should be equally poised on either side of the median plane. Any variation of an organ on one side of the plane, unless balanced by an exactly equivalent variation of the corresponding organ on the other side, would produce deviation of direction in movement. An increase of the median unpaired structures of the dorsal side would require some compensating change in the structure of the unpaired or paired structures of the ventral side, variations in size or form of the anterior end would require some modification of the structure at the posterior end, if the mechanical economy is to be maintained. There are many kinds of variation, therefore, which, in bilaterally symmetrical animals, would materially interfere with the chances of survival of the variant in the struggle for existence, but would not, at any rate to the same extent, interfere with the chances of a radially symmetrical sedentary or drifting animal.

Moreover, it can be understood that many kinds of sedentary and drifting animals have need of powers of variation and accommodation of form which are not required by animals that are active.

In most cases, sedentary animals begin life as free swimming or floating larvæ. These larvæ settle down in positions of various environmental conditions, and there they remain for life or perish.

Considerable powers of variations or plasticity in growth are therefore of immense advantage to a species for accommodation to the various and the variable conditions of the environment.

A sedentary animal that does exhibit considerable variability and accommodation has obviously a better chance of survival than one that is more rigid in its development and growth.

With an animal that is capable of vigorous muscular movements through its medium such variability has not the same essential value, for within limits it can escape from unfavourable environments and seek others that are better suited to its more rigid characters. Sedentary animals are either solitary or colonial in habit. Those that are solitary are almost invariably radially symmetrical, but those that are colonial, although showing radial symmetry as regards the individual of which the colony is composed, exhibit a great variety of form as regards the colony as a whole.

The study of the form of animal colonies is one of the most difficult, but at the same time most interesting, studies within the range of biological science, but at present very little progress has been made, and the solution of many of the problems that it affords is quite obscure. But we can see that in the formation of full-grown colonies there is a prevalent tendency to the construction of a radially symmetrical shape. Among the Madreporarian corals, for example, we find many examples of the solidly constructed Meandrinas, Siderastræas, etc., and of the ramose Pocilloporas and Seriatoporas that are spherical or hemispherical in shape. Among the Alcyonaria we find the club-shaped Alcyonium colonies, the mushroom-shaped Sarcophytums, the cylindrical Juncellas and the symmetrical bush-shaped colonies of such as species as *Chrysogorgia flexilis.* Among the sponges we find the spherical masses of Suberites, Tuberella and Tethea, and the horn-shaped Euplectella. Among the Polyzoa we find the beautiful funnel-shaped colonies of Retepora, the spherical masses of Cellepora and the disc-shaped colonies of Lagenipora.

In many other examples, however, the colony is not strictly radial in symmetry, as, for example, the flabellate forms of Gorgonia, the plicate forms of Madrepora and Millepora, and countless other varieties of branching and encrusting growth.

The general principle that seems to underlie the growth of these colonies is a tendency to assume a radially symmetrical form subject to modifications within a wide range, in adaptation to the variable conditions of the environment.

Thus, if the larva of one of these organisms settles down in shallow water in an open space where the necessary food may drift towards it from any point of the compass, it will, in the formation of its colony, send out branches of equal length in all directions, and thus assume a spherical shape; if it settles down in a narrow space between two massive rocks or corals, where the food from two directions is cut off, the shape of the colony will tend to become oval or flat. If the larva settles on a spot where the sea currents or wave actions are strong the branches will be strong and tend to anastomose; if it settles in deeper water where such are less powerful, the branches grow more delicately and do not anastomose. An interesting variety of such adaptation to conditions is seen in the genus Stylaster, one of the genera of hydroid corals that extends from shallow water to great depths of the ocean. The general form assumed by colonies of this genus is that of a fan, the main branches radiating from the base in one plane give rise to a large number of delicate branches which fill in the spaces between the main radii and frequently anastomose. As in all shallow water corals, however, the form of the colony is subject to great variations.

The polyps of Stylaster are arranged in cycles of five or six individuals—the Dactylozooids—with one larger individual—the Gasterozooid—in the centre of the cycle, and each of these cycles is protected by a calcareous cup which is called the calyx.

In the shallow-water species, such as *Stylaster eximius*, the calices are situated on the sides of the branches, that is to say, with the mouths of the cups facing in a direction parallel with the plane of the flabellum. In some of the deep-sea species, such as *S. umbonatus* and *S. minimus*, the calices are all situated on one surface of the branches, that is to say, with the mouths of the cups facing in a direction at right angles to the surface of the flabellum. It seems probable that the former arrangement is an adaptation to life in the strong varying currents and wave action of shallow water, and the latter to life in comparatively quiet water, with a gentle current flowing mainly in one direction, such as we find in some localities in deep water.

It is not necessary to refer to further examples of the great variability of external form, mode of growth, and method of ramification of the sedentary colonies of animals. They are well known to systematists interested in the various orders of the Porifera, Cœlenterata, Polyzoa, and Tunicata, but it must be remarked that the range of variability is by no means the same in all cases. In some genera, such as Millepora, the range is very wide. We find flat encrusting forms, plicate forms, fan-shaped branching forms, forms with thick anastomosing branches, and forms with delicate, not anastomosing, branches, of shrubby aspect. The anatomical structures of the polyps that build up all these manifold kinds of colony are found, on careful examination, to be identical.

In other genera, such as Meandrina, solid more or less spherical or oval colonies only are found; in others, such as Juncella, long cylindrical colonies; in others, such as Seriatopora, branching bushy colonies; and in many other genera flat laminate colonies following the contours of their support.

The shape assumed by a full-grown colony is not, therefore, entirely due to individual adaptation to the surrounding conditions. There is undoubtedly a genetic influence tending to produce a type of growth for each genus of a family, but this influence allows departures from the exact model of the type within certain ranges, which vary in extent in different genera.

Allowing for differences of this kind in the plasticity of growth, however, all the colonial and sedentary genera exhibit greater variation of shape than the active bilaterally symmetrical genera of the animal kingdom, and minor variations of shape cannot be used with any confidence for the diagnosis of species.

In the building up of the colonial organisation, the various methods of growth bring the individual polyps into a position in the water where they will receive during their vigorous lifetime the most abundant supply of food and, in some cases, of light. In such positions the food upon which the polyps feed will usually come, not from one direction only, but with greater or less frequency from all directions, and it is therefore to their advantage that the tentacles, of approximately equal length, should radiate from a common centre, where the mouth is situated. Upon the arrangement of the tentacles the whole symmetry of the body depends, and it is found that, whatever shape the colony as a whole may have assumed in adaptation to its environment, the radial symmetry of the tentacles, of the hard protective skeletal structures of these polyps, and of certain other organs of the body, is maintained.

But although the structure of the individual polyp and of the hard parts, such as the pores, calices, septa, cells (of Polyzoa), etc., are rightly regarded by systematists as more reliable than the form of the colony for the diagnosis of species, even these are liable to considerable variation in detail, according to their position on the colony.

The great variability that is observed in most of the species of colony-forming animals may be associated with their sedentary or stationary habit of life. It is only in a few cases, such as Pelagohydra, Cristatella, the Salps, and a few others that we find that the colony as a whole has the power of moving by its own efforts from place to place. In the order of the sea-pens, if we may judge from the character of the muscles, and from the direct evidence of observation of a few genera, every colony has the power of forcing its way into the sand by its own muscular movements. It seems probable that their movements are more powerful and rapid than the movements of any other colony of animal zooids.

Now, as Döderlein* has pointed out, the power of movement is correlated with plastic variability. When a species for any reason becomes less active in its movements—and he quotes *Bosmina coregoni* and *Daphnia hyalina* among others as examples of this—it tends to become much more variable. His view is summarised in the statement : "Die Höhe des Vagilität bei verschiedenen Thiergruppen muss in umgekehrtem Verhältniss Stehen zur Zahl der auf dem gleichen Gebiete vorhandenen adaptiven Formen."

Similarly, if in the evolution of a group of animals there has been an increase in power of movement (vagility), there has also been in all probability a diminution in the number of adaptive forms, or, in other words, a decrease in plastic variability. And the increase in power of movement is accompanied by a change from a previous radial symmetry to a more and more pronounced bilateral symmetry of the parts.

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* Döderlein, L. 'Zeitschr. für Morphologie,' vol. 4, p. 441 (1902).

Moreover, if we consider individual characters, we find that many of them, which are extremely variable in the forms which show radial symmetry, and are sedentary or show slight signs of movement, become rigid or less variable in the more active forms with bilateral symmetry. Characters which are determined in the former by external forces become genetic in the latter.

2. *On Symmetry and Variation in the Pennatulacea.*

The general statement that forms the substance of the introductory part of this lecture, namely, that radially symmetrical forms of animals are more variable than bilaterally symmetrical forms, is so difficult to prove absolutely, owing to a variety of complications in special cases, each of which requires separate consideration, that it would be of interest to discover whether in any one order which affords examples of genera showing both kinds of symmetry the radially symmetrical genera are more variable than those with bilateral symmetry. We find such an order in the Pennatulacea, an order which includes forms that are almost completely radially symmetrical, such as Veretillum, and forms such as Pennatula, that are bilaterally symmetrical.

At the time of the publication of the 12th edition of Linnæus' 'Systema Natura' (1768), eight species of the sea-pens were known, seven being described under the generic name Pennatula, and one (the deep-sea *Umbellula encrinus*) under the name Vorticella.

These species were included by Linnæus in his sub-class Zoophyta, a group of organisms which, notwithstanding the researches of Peysonnell and of John Ellis, were still regarded by Linnæus to be partly of the nature of animals and partly of the nature of plants: "Zoophyta composita Animalcula, in bivio Animalium Vegetabiliumque constituta . . . Stirps vegetans, metamorphosi transiens in florens Animal."

From the time of Linnæus and the final settlement of the Pennatulacea as an order of the Alcyonaria in the animal kingdom, little progress was made until the "Challenger" and subsequent scientific expeditions sent home their deep-sea booty. For most of the sea-pens are found to be inhabitants of deep or very deep sea-water, and it is only by the appliances carried by the great national deep-sea expeditions that they can be captured in large numbers and variety. The richest collection so far obtained is that of the

Dutch "Siboga" Expedition in the waters of the Malay Archipelago, which I have had the privilege to examine and describe.*

In the early days of the investigations of sea-pens, when one or two specimens from a single locality were all that a naturalist could examine, and the range of variation within a specific group could not be determined, there appeared to be a much greater discontinuity than actually occurs in nature, and in the course of time a large number of generic and specific names were suggested which have since been suppressed or submerged.

The result of the examination of the collection of sea-pens made by the naturalists of the "Siboga" Expedition and of other large collections in the British and Dutch Museums has led to the conclusion that many of the genera and species of previous writers are but local varieties of genera and species that have already been described, and further that the degree of variability is much greater in those forms that exhibit radial symmetry than in those which are bilaterally symmetrical.

In order to make this point clear it is necessary to call attention to one or two features of the Pennatulid colony.

The body of a sea-pen consists of a colony of trimorphic or quadrimorphic zooids, which in the most familiar genus Pennatula has the external form of a feather.

The first-formed zooid or "oozooid" becomes profoundly modified in the course of its development to form the rachis and quill (or "stalk," as it is called in the terminology of the Pennatulacea) of the feather and all the other zooids (with a few exceptions), which are formed by gemmination from the oozoid, are borne by the rachis. Of the secondary zooids one group—the autozooids—exhibit the typical alcyonarian characters and alone bear generative organs; and it is the arrangement of these autozooids on the rachis that gives us the external signs of the symmetry of the colony.

In a bilaterally symmetrical genus such as Pennatula the autozooids are arranged in rows on opposite sides of the rachis, leaving two broad tracks, extending from the base to the apex, free from autozooids. These two tracks on the rachis that do not bear autozooids are known as the dorsal and ventral tracks and they can be distinguished from one another by the order of succession of the autozooids in the rows. In a radially symmetrical genus such as Veretillum or Cavernularia, on the other hand, the autozooids are scattered quite irregularly all round the surface of the cylindrical rachis and there is no trace either of a dorsal or of a ventral track free from autozooids.

Between these two kinds of sea-pens we find a series of genera that are intermediate in character as regards their symmetry. In Echinoptilum, for example, the autozooids are distributed all over the rachis except along a short and narrow groove on one side.

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* Hickson, S. J., 'Siboga Expeditie,' "LXXVII. Pennatulacea," 1916.

In Sclerobelemnon there is usually a well-marked dorsal track free from autozooids, but in some specimens one or, more rarely, more than one autozooid occurs in the course of the dorsal track. And in this genus there is no trace of a ventral track. In Anthoptilum there is always a complete dorsal track but usually very little trace of a ventral track. And finally in Pteroeides, Pennatula and some other genera both tracks are always present and always free from autozooids.

Deeply imbedded in the tissues of the rachis and stalk there is usually found a long calcified rod, known as the axis. It is always present in bilaterally symmetrical genera, but it may be absent or incomplete in some of the radially symmetrical genera.

In the walls of the zooids and in the surface tissues of the rachis and stalk there are usually found numerous calcareous spicules. The shape and size of these spicules are usually regarded as of the greatest importance in the diagnosis of genera and species, but although they are of great value as accessory characters in recognising some forms, and particularly in the bilaterally symmetrical genera, they are so variable, both in form and size, in others that they may be most unrealiable for systematic work.

It is unfortunate that owing to the deep-sea habits of the sea-pens we know very little about their powers of movement and habit. Rumphius,* in 1705, stated that the *Sagitta marina alba* (probably a species of Virgularia), found at low tide on the shores of Amboyna, burrows deeper and deeper into the sand as the tide ebbs, and Darwin relates that the sea-pens (probably a species of Stylatula) found off the coast of Patagonia, "when touched or pulled, suddenly drew themselves in with great force, so as nearly or quite to disappear." The huge *Osteocella septentrionalis* of British Columbia is said to writhe like a worm when it is caught, and there are scattered observations that in an aquarium Pteroeides will bore with its stalk into the sand and draw itself upright. But these observations were all made on bilaterally symmetrical sea-pens and we are still without information, by direct observation, about the activities and habits of the radially symmetrical forms.

We know then from direct observation that the sea-pens, unlike other Alcyonarian colonies, can move through the sand in which they burrow and that in some cases these movements are rapid and powerful and therefore must be due to muscular contraction.

We do not know from observation that the movements of the bilaterally symmetrical forms are more rapid and more powerful than the movements of the radially symmetrical forms; but, judging from the arrangement and development of the muscles, it seems very probable that they are.

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* Rumphius, 'D'Amboinsche Rariteitkamer,' 1705.

The difference in variability between the radially symmetrical and the bilaterally symmetrical sea-pens may be seen in almost all the important characters upon which the classification is based.

The axis is present in all the bilaterally symmetrical Pennatulacea. In such genera as Pennatula, Pteroeides and Scytalium for example every specimen that has been examined has been provided with an axis extending from one end of the colony to the other. Moreover, in all the specimens of Pennatula and of Pteroeides that I have examined the axis is always circular in section, tapering at each end to a fine point, and in all the specimens of Scytalium and Funiculina the axis is four-sided (square with rounded angles in section). There seem to be no variations from these characters in any species. In some of the intermediate genera such as Umbellula and Virgularia, for example, the axis is complete but is sometimes four-sided and sometimes circular in section. In the classification recently suggested by Kükenthal the species of the genus Umbellula are arranged in groups according to the possession of a round or a quadrangular axis. In the genus Virgularia most of the species that have been described have a cylindrical axis, but in *V. rumphii* and in *V. gustaviana* the axis is sometimes cylindrical and sometimes four-sided. In the radially symmetrical Pennatulacea the axis varies greatly in length or may be absent. In the genera Actinoptilum, Echinoptilum and Renilla no trace of the axis has yet been found. Of these genera the last two named show signs of incipient bilateral symmetry but are, nevertheless, more closely related in their general anatomy to the Veretillidæ than to the higher forms of Pennatulacea. In the genus Lituaria one species (*L. australasiæ*) shows an incomplete axis, in the other two species the axis is complete but, as the number of specimens of these species that have been examined is as yet very small, it is quite possible that there is considerable variation in this respect in each of the three species. It is interesting to note too that in *L. phalloides*, according to Fowler, the complete axis is associated with an incipient bilateral symmetry. In the genus Veretillum the axis is very variable. In two species (*V. malayense* and *V. tenue*) the axis is said to be complete, but as only five specimens have at present been examined, it may be a variable character. In *V. cynomorium*, of which a very large number of specimens have been examined, the axis is incomplete and varies considerably in length.

In the genus Cavernularia, which is, perhaps, the most typically radially symmetrical of all the sea-pens, the axis is extraordinarily variable. Of this genus, five species have been described without any axis; in five species the axis is incomplete or variable, and in three species the axis is said to be complete. In the description of *Cavernularia habereri* from Japanese waters, Balss states that, on examining a number of specimens, some were found to have a short axis of variable length, and others to have no axis at all. As the axis in this species is found to be variable, even in specimens from the same locality, it seems probable that the length of axis cannot be taken as a sound specific character even in the diagnosis of a species of this genus.

As regards the shape of the axis in these forms, the evidence is, perhaps, not sufficient to justify any general conclusions. It has been described as round in section, square, square with fluted sides, and in one species at least (*L. hicksonii*) it is square above and round in section below.

The axis then seems to be a character which is variable both in length and shape in the radially symmetrical Veretillidæ, but attains to its full length in forms that show an incipient bilateral symmetry, as in *Lituaria phalloides*, and its full length and definite shape in the higher forms of Pennatulids, or to disappear entirely from all specimens that show an incipient bilateral symmetry, as in the genera Echinoptilum and Renilla. It is clearly an unreliable character for systematic purposes in the Veretillidæ, but may be a reliable character in other families.

The calcareous spicules that occur in the wall of the rachis and in the body wall and tentacles of the zooids have for many years been relied upon to afford good characters for the diagnosis of the species of the Pennatulacea, but the development of our knowledge of these characters shows that there are differences in the degree of variability of the spicules similar to those found in the case of the axis. In the genus Pennatula, for example, the spicules found in the rachis and zooids are always long, narrow spindles, showing three longitudinal curved flanges (the "dreiflügelig" spicule of Kükenthal). They vary in length according to position, and they vary in number and thickness according to position and according to the species, but they are always three-flanged. In the genus Pteroeides the spicules, although varying in length and thickness according to the position and species, are always smooth, long rods or spindles without the three flanges. Again, to take one more example from the higher bilaterally symmetrical genera, in the genus Scytalium the spicules are always very small, flat discs (about 0·05 mm. in diameter), round, oval, or dumb-bell-shape in outline.

In other families of bilaterally symmetrical sea-pens, we find examples, such as Virgularia, in which there are never any spicules in the rachis or zooids, and Stylatula, in which spicules are always present in a certain position in the colony. In another genus, Umbellula, with less pronounced bilateral symmetry, spicules are entirely absent in some species, but present and very variable in others.

In the radially symmetrical Veretillidæ the spicules are so variable that they afford very unreliable characters either for generic or specific diagnosis. In Veretillum itself the spicules of the rachis are nearly all thin, flat plates or rods, but their size and outline shape are so variable, even in a single preparation, that it is difficult to find any common characters. They may be of almost any size up to a maximum of about 0·25 mm. in length. Some are round or oval discs with serrate edges, some are oval or dumb-bell-shaped, divided by a line into two parts, technically known as twins, others divided into four parts by crossed line (the quadruplets), then there are straight rods, spindles, and others of quite irregular outline.

In a careful investigation of the *Veretillum cynomorium* of the Mediterranean Sea, Niedermeyer* finds that "Die äussere Form der Spicula ist ausserordentlich variabel, und man kann wohl sagen dass sich kaum zwei gleiche vorfinden."

The variability of these spicules in form and size is paralleled by their variability in number. In some specimens of a species the spicules are so scarce that they may be overlooked, in others they may be very numerous. Moreover, in some parts of the rachis of a single colony they may be crowded together, and in other parts very scarce.

In Lituaria we also find a great variety in the distribution and shape of the spicules. Many varieties of flat plates and rods are found, similar to those of Veretillum, but, in addition, we usually find a number of thick double-star or "capstan" spicules, which occur in no other genus of the order.

In the wide-spread genus Cavernularia most of the spicules in some species are straight, flat rods, but forked or branched forms and spindles are sometimes abundant. Kükenthal and Broch† have placed a species into a separate genus (Cavernulina) on the ground of the variability of its spicules, and state, "mit voller Sicherheit," that such a variation does not occur in Cavernularia. But this statement is not in accordance with the previous investigations of Kölliker‡ nor of Balss,§ who states, in his description of *C. habereri,* that the spicules are so extraordinarily variable that they are not adapted for species diagnosis.

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* Niedermeyer, 'Zool. Anz.,' vol. 43, p. 263 (1913).

† Kükenthal and Broch, 'Wiss. Ergeb. d. Deutschen Tiefsee-Expedition "Valdivia,"' 1911.

‡ Kölliker, 'Abh. Senckenb. Ges.,' 1870.

§ Balss, 'Abh. K. Bayr. Akad. d. Wiss.,' Supplementband, 1910.

A remarkable illustration of the variability of the spicules of this genus was obtained by the examination of four specimens obtained by the "Siboga" Expedition. These specimens were all dredged up at the same time in the anchorage at Amboyna, and, although they vary in length from 40 mm. to 113 mm., there can be no doubt that they belong to the same species. A preparation of the cortex of the rachis of the two larger specimens shows a dense felt-work of spicules, and, when these are teased out, it is found that the great majority of them are flat rods with round bifurcated extremities; but, in addition, there are many twins, triplets, and other varieties. In the two smaller specimens, however, the spicules are much less densely clustered, and the predominating type is a small, flat rod without bifurcated extremities. An even more remarkable illustration, however, was found in the examination of a number of specimens of *Sclerobelemnon burgeri* from the same dredging in the Molo Strait. This species, although it usually exhibits an incipient bilateral symmetry, is so closely related to Veretillum that some specimens are almost exactly intermediate in character between the two genera. The spicules of the rachis are, as in Veretillum, thin, flat plates with a great variety of outline; but the majority of them are irregularly round, oval, or dumb-bell-shaped, frequently divided by lines into twins, triplets, quadruplets, and multiplets, but rod- or spindle-shaped spicules are scarce. With so much variety in the spicules of a single specimen there was found great variation from one specimen to another. In one of two specimens from the same station, of approximately the same size, the spicules were few in number, and the larger ones 0·4 mm. in diameter, and in the other the spicules were numerous, and the larger ones only 0·04 mm. in diameter. This difference between the two specimens is so striking that, according to the general practice of systematists, they would undoubtedly be placed in distinct species, but fortunately there are 60 other specimens of various sizes from the same locality, and an examination of these shows that no two specimens are alike as regards the spicular armature, and that there are many intermediate cases between the two that were first mentioned. There can be no doubt therefore that in *S. burgeri* we have an example of a species in which the spicules are so variable that they cannot be regarded as being of any value for the separation of species.

The presence or absence of spicules in the expansible parts (anthocodiæ) of the autozooids has been used by some authors to distinguish genera from one another. Thus Kölliker distinguished the genus Clavella from Lituaria by the presence of spicules in the anthocodiæ of the former and their absence in the latter, but the only known species of the old genus Clavella resembles *L. phalloides* so closely in other respects that it cannot be separated generically from it. In a species described recently by Thomson and Simpson* from the Indian Ocean under the name *L. hicksoni*, there are no spicules in the tentacles. In a species described by Balss from Japan, under the name *L. habereri*, there are numerous spicules in the tentacles. In other respects the two species are so much alike that it is difficult to separate them, and, as regards the spiculation of the tentacles, we find an intermediate condition in specimens from the Malay Archipelago, in which spicules are occasionally present in the tentacles, but usually absent. In the genus Veretillum spicules are said to be present in the anthocodiæ of *V. cynomorium*, but are absent in that position in the other four species.

Similarly with respect to the presence of spicules in the cortex of the rachis. A genus (Policella) was established by Gray† in 1870, and more fully described by Kölliker in 1872. In Kölliker's description great stress was laid on the character of the absence of spicules in the rachis, to distinguish the genus from Veretillum. On making an examination of the Gray's type specimen, however, he found a considerable number of large spicules in the rachis. Marshall and Fowler‡ found no spicules in the rachis of two specimens of *P. manillensis* from the Indian Ocean, but a few calcareous bodies in that position in a new species they named *P. tenuis*. In a specimen from the Malay Archipelago, which in other respects closely resembles the *P. tenuis* of Marshall and Fowler, I could find no trace either of formed spicules or of calcareous bodies. With the failure of this character, the genus Policella has become merged with Veretillum.

No such wide variations as regards the variations in the distribution of spicules are found in the higher bilaterally symmetrical Pennatulids. In Pennatula, Pteroeides, or Scytalium, the presence of spicules in the rachis is a constant character; in Virgularia their absence is a constant character. In any one species of the species that have spicules in the rachis, spicules are either present or absent in the body-wall or the tentacles of the anthocodiæ. For example, spicules occur in the tentacles of *P. naresi*, but do not occur in the tentacles of any one of the many specimens of *P. murrayi* that I have examined. In *S. balssii* there are numerous spicules in the body-wall of the anthocodiæ and in the tentacles, but in *S. martensii*, of which I have had over a hundred specimens to examine, spicules are only found quite at the base of the body-wall, and never in the tentacles.

This character, however, is one that must be used with some caution for systematic purposes, even in the higher Pennatulids, as it is possible that differences may be found in some cases in the spicular armature of young and old colonies. Thus Grieg has shown that, in young colonies of *Funiculina quadrangularis*,* spicules are present in the tentacles of the young, but not in the tentacles of the old colonies. In other cases the spicules seem to be more numerous and widely distributed in the older colonies than in the younger ones.

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* Thomson and Simpson, 'Indian Alcyonaria,' Calcutta, Part II, 1909.

† Gray, 'Catalogue of Sea-pens, British Museum,' 1870.

‡ Marshall and Fowler, 'Trans. Roy. Soc. Edinb.,' vol. 33 (1887).

Moreover, if the spicules of any particular part of the rachis, such as the dorsal track, the calyx of the siphonozooids, the outer border of a leaf, or the tentacles be examined in a number of specimens of a given species, it will be found that they are constantly of the same form and approximately of the same size, although occasionally local variations are found in the number and size of the spicules in the zooids, due perhaps to local environmental conditions. An interesting example of such a local variation in the spicules was found in the examination of three specimens of *P. fimbriata* from the coast of Timor and two specimens of the same species from the Kei Islands. The species is an interesting one, as being intermediate in many respects between the two genera Leioptilum and Pennatula, and one of the distinguishing features given by previous authors of the genus Leioptilum is that spicules are confined to the margins of the leaves, whereas in Pennatula they are more evenly distributed throughout the whole leaf. In two specimens of *P. fimbriata* from the Kei Islands the spicules are confined to the margins of the leaf as in Pennatula, but in the three specimens from Timor these marginal spicules are supplemented by others, extending almost to the base of the leaf. The spicules of the Timor specimens, moreover, are much larger and more numerous throughout the colony than in the specimens from the Kei Islands. Many other instances, probably, could be found of local variations of the spicular armature of the higher Pennatulids, but such instances ought not to obscure the much more impressive fact of the constancy of these characters in specimens taken from a single locality, and the remarkable similarity, in most cases, as regards this character, seen in specimens from distant localities, when the higher Pennatulids are compared with the radially symmetrical Veretillidæ.

There is one more character frequently used in systematic treatises that may be briefly referred to before passing on to a more general statement. If a large number of specimens of a species of Pennatula from a single locality be carefully measured, it will be found that the ratio of the length of stalk to length of rachis is fairly constant. For example, in 38 specimens of *P. phosphorea*, var. *candida*, from the Mediterranean Sea, the ratio of stalk-length to rachis-length was about 1 : 1·5, the range extending to 1 : 1·9 and 1 : 0·8. In nine specimens of the same species, var. *variegata*, from the coast of Denmark, the range extended from 1 : 1·86 to 1 : 1·13. In 33 specimens of *P. pearceyi* from the coast of East Africa, the range extended from 1 : 1·7 to 1 : 3·2 (Kükenthal and Broch). In six specimens of *P. murrayi* from the coast of Timor, the range extended from 1 : 3·6 to 1 : 5·5, and in four specimens of the same species from the Kei Islands from 1 : 2 to 1 : 4. All the specimens of *P. phosphorea* were obtained in shallow water (150 metres), the specimens of *P. pearceyi* in deep water (693–1134 metres), the deep-sea forms in this case having a shorter stalk.

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* Grieg, 'Bergens Museums Aarbog,' 1896.

The specimens of *P. murrayi* from Timor were obtained in shallow water (112 metres), and those from the Kei Islands in deep water (310–397 metres), the deep-sea forms in this case having a longer stalk.

A consideration of these ratios and others that have been worked out leads to the conclusion that, even in the genus Pennatula, the stalk-rachis ratio is not a very reliable character for the determination of species, and, further, it seems to indicate that the rachis is much more variable in deep-sea species than in shallow-water species. Whether the relatively short stalk in specimens of *P. murrayi* from deep water is to be correlated with depth or with the character of the sea bottom is a matter that requires further investigation. Nevertheless, through all the conflicting evidence afforded by these measurements, it seems that the specimens of a given species living under the same conditions of depth and sea bottom have in this genus, at least, a ratio of length of stalk to length of rachis that varies within a small range from a common average, and this conclusion is confirmed by the measurements I have made of 30 specimens of *S. martensii* from the same locality off the coast of Timor, in which the length of the stalk was about 4½ times the total length of the colony. In the radially symmetrical Veretillidæ it is difficult to obtain trustworthy estimates of this ratio, because specimens seem to suffer more from contraction during preservation than specimens from the genera with a complete axis. Neidermeyer found, however, that in *V. cynomorium* no two species possessed the same ratio of length of stalk to length of rachis, and my own impression, from the study of this family, is that the character is much too variable, even in specimens from the same locality, to be of any value for systematic purposes.

Having now discussed in some detail the differences observed in the variability of certain individual characters, it is necessary to turn to the characters as a whole of the genera and species to ascertain if their study confirms the conclusion already indicated. It might be anticipated that if the individual characters in any one group are more variable than in another there would be greater difficulty in determining the generic and specific limitations in the former than in the latter, and that there should be, also, a greater discontinuity between genera and species of the former, that are less variable, than there is between the forms that are more variable.

This is exactly what we do find in the order of the sea-pens. In the higher groups with very pronounced bilateral symmetry there is seldom any difficulty in assigning a specimen to its proper genus. In the family Pennatulidæ, for example, there are four well known genera: Pennatula, Leioptilum, Acanthoptilum, and Scytalium. Pennatula stands out distinctly from the others in the character and distribution of the spicules, the arrangement of the spicules to form the so-called calyx teeth, in the shape and texture of the leaves, and in several other characters. The only difficulty in this case arises in the species *P. fimbriata*, in which, as previously mentioned, the arrangement of the spicules on the leaves is in some specimens intermediate between Pennatula and Leioptilum. Leioptilum, with its closely set leaves with thick margins, is also quite distinct, Scytalium with its minute flat spicules can always and at once be separated from the other genera, and Acanthoptilum with its fan-shaped arrangement of flanged spicules in the leaves and minute disc-shaped spicules in the calices is equally distinct. In the family Pteroeididæ, also the three genera Pteroeides, Sarcoptilum, and Gyrophyllum are quite distinct. Each genus is distinguished by several well-marked characters, and there are no intermediate forms.

In the Veretillidæ, on the other hand, the generic groups are very indistinct, as is particularly well shown in the instability of most of the generic names. Clavella has been merged with Lituaria, Policella with Veretillum, Sarcobelemnon, Stylobelemnon, Fusticularia, and Cavernulina, with Cavernularia, and the characters which are used for the separation of the four remaining genera, Lituaria, Veretillum, Cavernularia, and Actinoptilum, are admittedly very variable and unsatisfactory. Lituaria differs from Veretillum only in the presence of the capstan or double-star spicules, and in some specimens of Lituaria these spicules are not very abundant, and are always supplemented by flat plate-like spicules similar to the prevailing type in Veretillum. Veretillum is closely related to Cavernularia, although the autozooids are usually larger in the former than in the latter, and the spicules of Cavernularia are usually rod-shaped or forked. Actinoptilum is separated from Cavernularia by the presence of verrucæ for the autozooids, a character which is very variable, and depends to a considerable extent upon the method of preservation.

Moreover, there are connecting links between the Veretillidæ and the related families with incipient bilateral symmetry. A few rare specimens of Echinoptilum without any ventral track bridge the gap between the Echinoptilidæ and the Veretillidæ, and some specimens of *Sclerobelemnon burgeri* can only with difficulty be separated from Veretillum.

As regards the separation of generic groups into species, there are throughout the group endless difficulties owing to the want of agreement among the authorities as to a working plan for recognising the difference between "species" and "variety." These difficulties are the same as those that are met with in nearly all the classes both of the animal and vegetable kingdoms, and do not call for special comment. Nevertheless, in the study of a large collection of Pennatulacea a very great difference is observed in the facility with which specimens of the higher families and of the lower families can be confidently assigned to their specific groups.

In the genus Pennatula, for example, some of the species, such as *P. grandis, P. fimbriata, P. murrayi, P. naresi*, and *P. phosphorea,* are quite distinct, and can be recognised by several well-defined characters. In Scytalium three of the six species are well defined, and of the others our knowledge is not yet sufficient to enable a judgment to be given as to whether they are well-defined species or not.

Even in Virgularia, a widespread genus of which a very large number of species and specimens have been examined and described, several of the species can be easily recognised on careful examination. In the Veretillidae, however, difficulties of arranging specimens in defined specific groups are practically insuperable. When a large number of specimens are obtained from one locality they are found to exhibit so much variation that overlapping of several described species may be discovered, and the present-day classification is extremely unsatisfactory. Moreover, in the genus Echinoptilum, with incipient bilateral symmetry, the characters that are used to separate the six species that have been described are so unsatisfactory that it seems quite probable that these species will prove to be only local varieties of one widely distributed but very variable species. It would take too much time and space to analyse more fully the specific grouping of the order. Such an analysis would involve the discussion of some exceptional cases such as that afforded by the genus Pteroeides in which the species appear to overlap as in the radially symmetrical forms. It is only to be taken as an expression of opinion based on a long study of a very large collection of specimens of sea-pens that with the evolution of a bilateral symmetry and increased power of movement the specific groups tend to become better differentiated.

3. *On the Classification of Sedentary Animals.*

The difficulties that are found in separating the radially symmetrical Pennatulacea into defined specific groups are met with even in a more pronounced degree in the study of the corals, in the sub-orders of Alcyonaria, and in some other groups of sedentary animals; and it may even be suggested that, as our knowledge of the range of variation increases, the conclusion will be reached that, in some cases at least, the evolution of those discontinuous groups which are commonly recognised as "species" does not occur in nature. In some of the genera of sedentary Cœlenterata, such as Millepora, Tubipora, and Stylaster, and in some sedentary Foraminifera such as Sporadotrema and Polytrema, with a very wide geographical distribution in shallow water, very careful examination of a large number of specimens and an analysis of all possible characters that would be used in classification show very little if any evidence of the existence of discontinuous specific groups. There are differences between specimens from different localities in the mode of branching, in colour, in the size of the zooids, and in the arrangement of the zooids on the colonies, but these characters are found to be so variable when a number of specimens are examined from the same locality that it is impossible to use them in the definition of species. Similarly Bernard, in preparing his monumental catalogue of the Madreporaria in the British Museum, found the difficulties of maintaining the old or establishing new species so great that he abandoned the orthodox binomial system and grouped the specimens according to their growth forms and geographical distribution, "The task of establishing genetic species," he writes, "is practically hopeless in such a case as Porites."* And Wood Jones† after a careful study of the different forms assumed by the corals on a reef writes, "There is no doubt that a great number of our museum-made species are mere vegetative varieties, produced in response to the demands of the environment."

Nor are the difficulties of determining species confined to the sedentary Cœlenterata, as we see, for example, in the statement made by Gregory‡ in his introduction to the catalogue of the Jurassic Bryozoa, that he came reluctantly to the conclusion "that there are no true genera among Cyclostomata but only certain convenient artificial groups of species." It might be urged in reply to these expressions of opinion, and to many others of a similar kind that have not been quoted, that, unless the anatomy of the zooids and their connections with one another are as carefully studied as the skeletal structures, the conclusions are based on insufficient evidence. There are many practical difficulties in the investigation of a large number of examples of the soft and perishable tissues of these organisms, difficulties which are insuperable in the case of the extinct genera; but it seems improbable that a well-marked discontinuity in the structure of the mesenteries, tentacles, body wall and other parts of the zooids would leave no corresponding impressions on the hard parts as they are built up.

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* Bernard, 'Catalogue of Madreporaria, Brit. Mus.,' V, p. 27 (1905).

† Wood Jones, 'Proc. Zool. Soc.,' 1904, p. 555.

‡ Gregory, 'Catalogue of Jurassic Bryozoa, Brit. Mus.,' 1896, p. 21.

A thorough investigation of the soft parts is, however, most desirable, whenever it is possible, to test the accuracy of the conclusions derived from the study of the hard parts, and we have already a valuable contribution to our knowledge in this direction in the investigations of Mr. Matthai on the anatomy of certain Astræid corals. Mr. Matthai* gives a list of 10 different characters of the hard parts of these corals which are usually regarded by systematists as of value in their schemes of classifications and comes to the conclusion that none of them have any constant value "and therefore the distinctions based upon them would be arbitrary," but he finds that "any species whose limits had once been settled by the study of both polyps and hard parts can be recognised later from the hard parts alone." With the admitted variability of the hard parts, it is not clear how this recognition of the species can be made; but it does not appear to me that the author of this valuable contribution to our knowledge has been able to prove from his investigations that the soft parts of these corals are less variable than the hard parts.

In an examination I made some years ago of both the hard and soft parts of a large number of specimens of the genus Millepora, obtained from many different localities both in the East and in the West Indies, I† found that the soft parts gave no assistance in the determination of species. But what may be true of one genus or family may not be true of another, and it is quite possible that in some kinds of sedentary animals, and more particularly in some forms of floating and drifting animals, there may be true genetic species. The causes that have brought about, in the course of evolution, the discontinuity which, in the bilaterally symmetrical animals, enables us to recognise distinct species are so numerous and involved that it would be presumptuous to assert that they can never affect animals that do not move about by their own muscular effort. In fact the existence of specific groups in the higher plants, every bit as well defined as in the higher animals, should be sufficient to convince any one that causes leading to discontinuity may affect all kinds of sedentary organisms. In the course of my systematic work on Cœlenterata, I have come across several instances in which there seem to be distinct specific differences between groups of specimens belonging to the same genus. For example, the two species *Alcyonium digitatum* and *A. glomeratum* appear to be quite good species. They are found close together in some parts of the waters of the British coast (*e.g.*, the south coast of Cornwall), and the first-named species, which is by far the most abundant, exhibits a wide range of variation in form, colour, and spiculation. Nevertheless, I have not yet found a single variety of *A. digitatum* among the many hundreds I have examined that could possibly be mistaken for *A. glomeratum*, or any other species of the genus. But even if it be established that specific discontinuity does occur in some genera of sedentary animals, it does not follow that such discontinuity occurs in all or even in a majority of them, and the evidence, so far as it goes at present, tends to show that it is exceptional rather than universal.

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* Matthai, 'Trans. Linn. Soc.,' vol. 17, p. 1 (1914).

† Hickson, 'Proc. Zool. Soc.,' 1898.

In attempting to make a scientific classification of any group of animals it is found that the characters of the greatest value are those that are least variable; but characters that are very variable in one family or genus may be much less variable in another family, and it is therefore necessary, before a suggested scheme of classification can be regarded as stable, to ascertain the range of variation of the characters it is proposed to use.

Thus in the shrimp Hippolyte the character "colour" is so variable that it cannot be used in specific diagnosis, but in the crayfish Astacus (Potamobius) the red colour of the chelæ of *Astacus flaviatilis*, and the pale colour of the chelæ of *A. pallipes* are invariable characters, supported by others, for distinguishing the species.

In the sea-anemone *Metridium marginatum* only 33 per cent. of 131 adult specimens exhibited the arrangement of mesenteries which is regarded as normal for the species, but in *Actinia equina* only 4·24 per cent. showed variations from the normal arrangement of the mesenteries. In *A. equina*, therefore, the arrangement of the mesenteries is a much more reliable character for specific diagnosis than it is in *Metridium marginatum*.

Some years ago* I suggested the use of the term "plastic" for characters that are variable within the species and "rigid" for characters that are fixed, or show only a small percentage of variations. The term "plastic" suggests that the characters are to some extent moulded or modified in the course of their development by external forces, and that the exact form that they exhibit in the adult must be due in large measure to the environment. There seems to be no doubt that this is true of many of the plastic characters of sedentary animals, although it may be difficult to prove to be true in the case of some of the variable characters of the higher bilaterally

* 'Reports of British Association,' 1903, p. 680.

symmetrical animals. But characters that are rigid and are not, therefore, subject to fluctuations caused by external forces must be genetic, they are characters transmitted as such by heredity. It is on the rigid characters, therefore, rather than on the plastic characters, that we must rely for a scientific basis for the diagnosis of species. But in the groups of radially symmetrical animals with which we have been dealing more particularly in this lecture, it has been shown that there is a larger proportion of plastic characters than in the bilaterally symmetrical animals, and, moreover, that many of the characters that are plastic in one genus may be rigid in another. The first step therefore in a scientific classification of these animals is to find out the degree of plasticity of the characters it is proposed to use for the diagnosis of each genus and species. Until this is done, the classification can only be regarded as provisional. But the practical difficulty that so often occurs in systematic work is that the number of specimens available is so small that no reliable estimates can be formed of the plasticity of the characters they exhibit, and the question arises, What should be done with isolated specimens of which the plasticity of the characters cannot be determined? It seems to me that if a new specimen differs from previously described specimens of a known species only by one character that is known to be very variable in the genus, it should not be regarded as a new species, but be regarded as a plastic variety of the nearest known species, and if it is desired to call attention to some peculiarity of the plastic form, an additional name should be given to it to indicate this peculiarity. In order to avoid the term "variety," which is so frequently applied in higher animals and plants to genetic variations, I have used the term "facies" to signify a variety which is probably purely plastic in character. Thus, in *Millepora alcicornis*, I have used the expressions facies *ramosa*, facies *plicata*, facies *verrucosa*, etc., for various forms of growth or surface markings, the peculiar features of which are almost certainly caused by the external environment; and in the Foraminifer *Sporadotrema cylindrica* I have used geographical terms, such as facies *providentiæ*, facies *amirantiæ*, etc., for specimens differing from one another in plastic characters that cannot be easily described by a single word.

To use either of the terms "variety" or "sub-species" in these cases would be entirely misleading, for, as they are commonly used with reference to both the higher animals and plants, they imply genetic differences. Thus, an albino mouse or an albino stock is a variety that breeds true. Mammals with long hair, instead of the short hair that is normal for the species, produce offspring that are long-haired, and plants that have cut leaves instead of the entire leaf that is normal for the species produce offspring with cut leaves. But, so far as we can judge from circumstantial evidence, the offspring of a plicate *M. alcicornis* or a verrucose *M. alcicornis* would only produce plicate or verrucose offspring if the external conditions of their growth were the same as those of the parents.

The advantage of using some such word as "facies," instead of "variety" or "sub-species," to express these plastic variations in the sedentary forms of animal life is conclusive, but I would specially urge its adoption because of the tendency shown by some systematists, weary perhaps of the number of different forms of their specimens, simply to mention the name of the species to which they refer them without description. In this way a great deal of valuable information about the plasticity of species is withheld. The tendency there is to provide distinct specific names for local environmental forms has undoubtedly led to a great confusion in our system, and this confusion has, in its turn, tended to discourage systematic work in many groups of sedentary animals. New species are founded, and in a few years merged with others. However carefully the descriptions are made, there is little prospect of the system proposed remaining stable for any length of time, and the result is that it is becoming increasingly difficult to get any one to undertake a systematic description of a large collection of these groups. The advantage of the system of proposing new specific names for specimens differing from others only by certain plastic characters, however, must be acknowledged. It has at least placed on record detailed facts regarding these characters, and provided many excellent figures for future investigations. By the system I have suggested of using the species in a more comprehensive way, and describing the "facies" as frankly a local environmental form, there may be, I hope, a prospect of the revival of detailed analysis of collections.

4. *Evolution of the Pennatulacea.*

In the study of the order of the sea-pens, we find a series of forms showing at the one end of the scale almost complete radial symmetry, and at the other end a well marked bilateral symmetry. Moreover, as I have shown, it is among the radially symmetrical that we find that overlapping of genera and species, due to the wide range of variation of plastic characters, which makes systemic work so difficult, and among the bilaterally symmetrical forms that we find, on the whole, well defined genera and species. The question arises, then, whether this series represents the general outlines of the evolution of the order, *i.e.* from radial symmetry to bilateral symmetry, or *vice versâ*, or whether a centrally placed form such as Protoptilum represents the most primitive sea-pen from which the radially symmetrical forms have been derived by degeneration on the one hand, and the higher bilaterally symmetrical forms by differentiation and specialisation on the other. In the earlier writings of Kölliker, Studer, Wilson, Jungersen, and Bourne, the view is expressed that the Pennatulacea are derived from a bilaterally symmetrical stock, and such genera as Bathyptilum or Protoptilum are regarded as the most primitive genera of recent sea-pens.

It is to Kükenthal* that we owe the first definite suggestion that it is the radially symmetrical Veretillidæ that are the most primitive, and with his view I am in agreement.

The principal reasons in favour of Kükenthal's view may be summarised as follows:—

(1) The general structure of the colony of the radially symmetrical Pennatulacea is simpler than that of the bilaterally symmetrical ones. In the latter we find the two kinds of zooids, autozooids and siphonozooids, distributed all over the surface of the rachis without any definite arrangement in rows or leaves. It is difficult to believe that, in the evolution of the sea-pen from its Alcyonacean ancestry, there could have arisen by some great mutation that very definite and orderly arrangement of these zooids on each side of the rachis, that we find in such genera as Pennatula and Scytalium, and still more difficult to conceive a reason for a subsequent change of this arrangement unless accompanied by some very definite change of habit.

(2) In passing through the series leading from radially to the bilaterally symmetrical sea-pens, we find an increasing differentiation in the structure of the colony. Such definite organs as the radial canals of Virgularia and Osteocella, the specially differentiated zooids, which I have called "mesozooids," of Pteroeides and some species of Pennatula, and that peculiar type of spicule known as the three-flanged or "dreiflügelig" spicule, do not occur at all in the Veretillidæ, nor in any known genus of the other groups of Alcyonaria.

(3) The axis of the higher Pennatulacea is a skeletal structure which has no homology with any structures found in the other orders of Alcyonaria. It must have arisen within the Pennatulid stem as an organ adapted to the needs of a free colony, with an elaborate and powerful set of muscles. We should expect to find, therefore, this structure to be better developed in the higher forms with the more powerful muscles than in the more primitive forms, in which the muscular system is not so well developed. If we accept the view that the radially symmetrical forms are the most primitive, this is exactly what we do find. As already mentioned, it is only in

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* Kükenthal, W., 'Verhandl. VII. Internat. Zool. Congress. Graz,' p. 563 (1910).

the genera Cavernularia (some species), Actinoptilum, Echinophilum, and the aberrant Renilla, that we find that the axis is absent, and in Cavernularia (some species), Veretillum, and Lituaria that we find it incomplete.

(4) In the study of the Veretillidæ, Kükenthal discovered that the system of endodermal canals in the rachis has a closer resemblance to that of the other Alcyonacea than that of any of the higher sea-pens.

(5) In this connection, a small piece of evidence, but not an unimportant one, is found in the genus Lituaria, a genus closely allied to Veretillum, but occasionally showing incipient bilateral symmetry in the presence of a short groove on one side of the rachis free from autozooids, similar to the groove seen in Echinoptilum, and more rarely in Actinoptilum. In this genus only, we find. interspersed with other spicules of the types found in other Veretillidæ, a number of thick spicules of the shape of a dumb-bell, with sharp tubercular spines standing from each side of the swollen extremities. This type of spicule, known as the twin star ("doppelsternige," Kölliker) or "capstan" spicule, is not found in any other genus of the Pennatulacea. but is a common type in the Alcyonacea. This fact is significant, because the Pennatulid spicules, except in Lituaria, are quite different to those found in the Alcyonacea. The thin flat flakes of Veretillum, the smooth flat rods of Cavernularia. the smooth cylindrical rods of Umbellula and Pteroeides, and more particularly the remarkably specialised three-flanged spicule of Pennatula and several other genera, are quite peculiar to the Pennatulacea, and could not be confounded with any type of spicule found in the other Alcyonaria. The conclusion seems to be inevitable that the types of radially symmetrical Pennatulacea are not degenerate, but do represent the nearest approach to the ancestral type of which we have any knowledge.

The evidence in favour of the view that the radially symmetrical Pennatulacea are the most primitive appears to me so strong that it is worth while to consider how they arose from the Alcyonacean stock. Such consideration must necessarily be purely speculative, because at present no species has been discovered that can be regarded as intermediate in structure between the Pennatulacea and the other orders of the Alcyonaria and because the ontogeny of the Pennatulacea so far as it is known does not really shed any light on the matter. There seems to be little doubt that the ancestral form of the sea-pens was colonial in habit. At any rate there is no evidence derived from embryology or morphology to show that the origin of the stock dates back to the original solitary Alcyonarian polyp. If we are justified in making this assumption, as all previous writers have done, there are two hypotheses as to the further evolution. Either the Pennatulacea are derived directly from a sedentary Alcyonarian colony or indirectly through a floating or drifting stage. In either case the ancestry must have been radially symmetrical.

It seems probable from the little knowledge we possess of the natural history of the sea-pens that they are all capable of boring into the sand or mud at the bottom of the sea by the muscular movement of the stalk, and the difficulty of deriving them directly from the sedentary ancestry is that the body wall of such Alcyonarians is not provided with muscles capable of any such movements. There must have been between the absolutely sedentary ancestry and the more active burrowing Pennatulid an intermediate stage with some powers of muscular movement, and it may be suggested that this stage was a floating or drifting colony. The transition from a sedentary to a floating habit is not difficult to understand. The feeble musculature of the endoderm of Alcyonarians, which is used for contracting the cœlenteric cavities under certain unfavourable conditions, such as removal from the sea-water or exposure at low tide, could readily be adapted to slow pulsations sufficient to keep a colony afloat in running water and particularly so if it were supplemented by the ciliary action of the ectoderm. Moreover, an Alcyonarian showing a dimorphism of the zooids such as we find in Sarcophytum and Anthomastus, in which a flow of water through an elaborate plexus of canals in the substance of the colony is produced by the action of the siphonozooids, would be a more favourable form for adaptation to such a pelagic mode of life than the heavier monomorphic forms. In all the sedentary Alcyonaria about which we have information on the point the cilia supported by the ectoderm cells of the larva are lost when the fixation takes place. There is no record of a ciliated ectoderm covering the colony in any species. In the Pennatulacea, on the other hand, Kölliker* originally pointed out, there are certainly patches or tracts of cilia on the ectoderm of the rachis, although they appear to be absent on the stalk. My view, therefore, is that there was a stage in the evolution of the Pennatulacea when the colony became free from its sedentary habit and was dimorphic and ciliated. If this transition actually occurred, it would not be exceptional in the animal kingdom. The remarkable hydrozoan *Pelago hydra*, discovered by Dendy, was undoubtedly derived from a sedentary ancestry, and there is good reason to believe that the Salps and Doliolum were independently derived from sedentary Tunicates. It is unlikely that in this stage the axis was developed, as a heavy skeletal structure of this character would be of little use for the attachment of muscles such as would be used for pulsating movements, and its weight would impair the flotation power. The shape of the body, in conformity with other floating forms, would probably have shown a perfect radial symmetry, and, from the ancestral history, it would have probably had an outline like a top or pear. Without referring to certain changes of internal structures, which it is much more difficult to understand, we may suppose that, at the time this intermediate form was assuming the full Pennatulid characters, it was in form not unlike a Cavernularia, and succeeded, at times, in obtaining an insecure foothold in the sand. This suggestion is supported by the fact that *C. malabarica,* as related by Fowler,* is the only species of the order that has been found washed ashore in great numbers after a storm.

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* Kölliker, 'Anat. System. Beschreibung der Alcyonarien,' p. 424 (1872).

The habit and structure of the Pennatulid stock being thus established, the further evolution followed the main lines of increased powers of deep, rapid burrowing in the sand, accompanied by a completion of the development of the muscles and a gradual change to an almost complete bilateral symmetry of the colony as a whole.

This conception of the evolution of the Pennatulacea, which I have ventured to bring forward, seems to me to give a satisfactory explanation of two difficulties that are met with in the alternative hypothesis of a bilaterally symmetrical ancestry. It is very difficult to understand why a bilaterally symmetrical colony provided with an axis and with powers of burrowing deeply in the sand should lose the axis and become radially symmetrical. It has been suggested that the Veretillidæ are degenerate, but I cannot see that there is a shadow of evidence to support this view. They are not parasitic, sedentary, nor cryptic in habit, and there is no reason for supposing that in any structural characters they show signs of retrogressive evolution. The only ground for the assertion is that they show greater variation than the bilaterally symmetrical families, but, although it is undoubtedly a fact that degenerating organisms and structures are more variable than others that are not degenerating, it does not follow that, because organisms or structures are very variable, they are consequently degenerate or degenerating.

As I have attempted to show in the earlier part of this lecture, the great range of variation seen in the radially symmetrical Pennatulacea is to be associated not with the idea of degeneration, but rather with their feeble powers of movement and their radial symmetry.

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* Fowler, G. H., 'Proc. Zool. Soc.,' 1894, p. 376.

*Bactericidal Properties Conferred on the Blood by Intravenous Injections of Diamino-Acridine Sulphate.**

By C. H. Browning and R. Gulbransen.

(Communicated by Prof. R. Muir, F.R.S. Received August 11, 1917.)

(From the Bland-Sutton Institute of Pathology, Middlesex Hospital.)

Attempts to achieve "internal disinfection," that is to say, to kill organisms in the body of infected animals by means of drugs, have hitherto afforded little promise of success in the case of the common pathogenic bacteria, the only exception being the action of ethyl-hydrocuprein in pneumococcus infections, discovered by Morgenroth with his co-workers, Levy and others. The reasons for the failure are probably to be attributed mainly to two facts; in the first place, antiseptics in general enter into combination with proteins of the tissues and body fluids, either by a process of physical adsorption or by the formation of chemical compounds. In either case, the usual result is that the bactericidal action of a substance, as determined in a watery medium, is greatly reduced by protein-solutions, *e.g.*, serum. Secondly, the majority of chemical antiseptics are general protoplasm poisons, and exert on mammalian tissues a degree of toxic action equal to, or greater than, that which they exhibit towards micro-organisms; hence these substances prove lethal for animals in doses which are insufficient to confer bactericidal properties on the body fluids.

The classical example of such failures was afforded by mercuric chloride, which Koch employed in the hope of treating effectively anthrax septicæmia in animals. Ehrlich and Bechhold investigated compounds which were more potent antiseptics than any hitherto known, among the most active being tetrabrom- (and chlor-) ortho-biphenol; they found, however, that the bactericidal properties of these substances were also greatly reduced when the organisms were suspended in a serum medium. Thus, tetrachlor-ortho-biphenol in a concentration of 1 : 320,000 prevented the growth of diphtheria bacilli in bouillon, whereas in serum growth occurred in the presence of 1 : 10,000 of this reagent. Similarly we have found that the dose of perchloride of mercury which is required to inhibit completely the growth of *Staphylococcus aureus* or *B. coli* in serum is 100 times greater than that which produces this effect in watery medium containing a small amount of nutrient peptone (0·7 per cent.).

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* We are indebted to the Medical Research Committee for a grant towards the expenses of this work.

Browning and Gilmour, while investigating relationships between constitution and bactericidal action among basic benzol-derivatives, found that diamino-acridine was more powerfully bactericidal in the presence of serum than in ordinary peptone-water-agar medium. Subsequent observations by Browning, Gulbransen, Kennaway and Thornton have confirmed and extended this result; it has been found that a number of diamino-acridine derivatives with substituted methyl-groups either in the amino side-chains or in the benzol rings, or in both situations, *e.g.*, the dye acridine yellow, are all enhanced in their bactericidal action by serum. This is likewise the case with Benda's compound, 3 : 6-diamino-10-methyl-acridinium chloride.

In the presence of serum this group of substances constitutes the most potent bactericidal agents known, and the property of being enhanced in this activity by serum, so far as we are aware, is shared by no other type of chemical compound which has been investigated. On account of this property, together with the fact that they are comparatively non-toxic to mammalian tissues, and devoid of inhibitory effect on phagocytosis, diamino-acridine salts (sulphate and chloride) and diamino-methyl-acridinium chloride have been recommended as therapeutic substances for local application in the treatment of bacterial infection in wounds. Their bactericidal action is slowly progressive and the maximum effect is attained only after a considerable time, thus concentrations of these substances which at first merely inhibit proliferation of the organisms ultimately prove lethal; in this respect they differ from such substances as phenol, mercuric chloride, and sodium-toluene-para-sulphochloramide. Thus, if mixtures of serum with varying proportions of any of the latter compounds are inoculated with a suspension of a culture of living micro-organisms, such as *Staphylococcus aureus* or *B. coli*, and are placed in the incubator at 37° C., it is found on making subcultures at intervals, that if a given concentration of antiseptic has not proved lethal in two hours, its presence has little effect in preventing the occurrence of active multiplication of the organisms subsequently.

On the other hand, with the acridine compounds the effect in two hours is very slightly greater than with mercuric chloride, but the concentration which proves lethal in 24 hours is only a tenth to a twentieth of the lethal concentration of mercuric chloride. Thus, it was found that a concentration of 1 : 10,000 of mercuric chloride killed these organisms in serum in two hours, but with a concentration of 1 : 20,000 the bacteria were still alive after 24 hours and had multiplied actively. With diamino-methyl-acridinium chloride, however, the lethal concentration after two hours was 1 : 20,000, and after 24 hours a strength of 1 : 100,000 and 1 : 200,000 had killed *B. coli* and *Staphylococcus aureus* respectively; diamino-acridine sulphate is similar in its action. The relative lack of toxic effect on leucocytes is shown by the fact that when a volume of human "leucocyte cream" along with a volume of human serum, the mixture containing 1 : 10,000 of the acridine derivatives, is incubated for two hours at 37° C., and staphylococci are subsequently added—time then being allowed for phagocytosis to occur—an estimation of the phagocytic count yields over 50 per cent. of the number of cocci ingested by leucocytes subjected to similar conditions except that the dye was omitted from the mixture; on the other hand, 1 : 10,000 of mercuric chloride reduces the phagocytic power to a much greater extent. Now in the case of the mercury salt in serum, this concentration represents practically the limit beyond which effective antiseptic action does not take place; on the other hand, the acridine compounds in 1 : 10,000 dilution in serum are powerfully antiseptic and ultimately prove lethal to the organisms.

Experiments have shown that of the two substances diamino-acridine sulphate* is the more suited for direct injection into the blood stream, as it is less toxic and has less agglutinating action on the red blood corpuscles than diamino-methyl-acridinium chloride. Accordingly, the present investigation was undertaken with a view to ascertaining whether it was possible to render the blood serum antiseptic without at the same time damaging the health of an animal treated in this fashion; the results have shown that this aim could be realised.

The following preliminary observations, the results of which are shown in the Table, served to determine the relative toxicity of diamino-acridine sulphate and diamino-methyl-acridinium chloride, and also their bactericidal power for *Staphylococcus aureus* and *B. coli*.

Substance.	Maximum non-lethal dose for a normal 20-grm. mouse.	Bactericidal concentration for			
		Staph. aureus in		*B. coli* (Escherich) in	
		0·7-per-cent. peptone water.	Serum.	0·7-per-cent. peptone water.	Serum.
Diamino-acridine sulphate...	grm. 0·003	1 : 20,000	1 : 200,000	1 : 4000	1 : 100,000
Diamino-methyl-acridinium chloride	0·0006	1 : 20,000	1 : 200,000	1 : 1300	1 : 100,000

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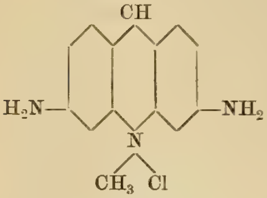
* We have pleasure in expressing our indebtedness to Drs. Barger and Ewins, of the Department of Biochemistry and Pharmacology of the Medical Research Committee, for their kindness in preparing for us the supply of this substance which we required.

Method of the Tests.—The toxicity for mice was determined by injecting watery solutions subcutaneously, the dose being so arranged that a 20-grm. mouse received a volume of 1 c.c.; to animals of other weights corresponding volumes were given, but mice not exceeding the limits of 15–25 grm. were selected for the tests.

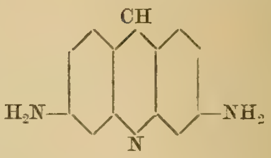
The bactericidal concentration was found thus: The substance to be tested, in a volume usually not exceeding 0·1 c.c., was added to small test-tubes containing 1 c.c. of the culture medium, which consisted in one series of 0·7 per cent. of peptone water, and in the other of undiluted serum, usually from the ox (previously heated at 56° C. for an hour), and then 0·1 c.c. of a 1 : 20,000 dilution in saline of a peptone water culture (previously incubated for 24 hours at 37° C.) was added. A control was made with peptone water or serum without antiseptic; one loopful of this mixture, when stroked immediately on agar, yielded about twenty colonies of *Staphylococcus* or *B. coli.* The tubes were then placed at 37° C., and were examined at the end of 24–48 hours, in order to determine the concentration of antiseptic which killed the organisms introduced; the development of turbidity, of course, indicated the occurrence of definite proliferation of the bacteria, but subcultures were made also on agar and in peptone water. The results of both methods of subculture corresponded in general, but it was sometimes found that cultures containing antiseptic which showed no turbidity after incubation, and in which, therefore, little or no multiplication of organisms had occurred, still contained living bacteria.

It is to be noted that we selected the quantity of bacteria employed for the inoculation dose because, when added to the standard volume of fluid used in our tests (1 c.c.), one loopful of the mixture yielded a convenient number of colonies (about twenty) for estimating subsequent increase or decrease. But the employment of 0·1 c.c. of undiluted culture, *i.e.* a 20,000-fold dose, required for sterilisation a concentration of antiseptic only $2\frac{1}{2}$–5 times greater than that recorded above.

Diamino-methyl-acridinium chloride was prepared by Benda for Ehrlich and was found to possess very marked curative properties for experimental trypanosome infections, hence the name "trypaflavin" was applied to it. The remarkable properties of this substance and other acridine compounds as bactericidal agents, so far as we know, was not suspected. It is of interest, in regard to the relationship existing between chemical constitution and therapeutic action, that the trypanocidal property depends greatly on the presence of the methyl-group attached to the nitrogen atom. This is implied, though not expressly stated in Benda's work.



Diamino-methyl-acridinium chloride.



Diamino-acridine (base).

We have investigated the question in the case of mice infected with *Tr. rhodesiense*.* The mice were inoculated by subcutaneous injection of a dilution of richly infected blood. After several days, when parasites were readily found in their blood, a series of animals received varying doses of diamino-methyl-acridinium chloride and diamino-acridine sulphate. The maximum dose of the acridinium compound tolerated by infected animals (0·0003 grm.) caused disappearance of the parasites from the blood for a number of days as a rule, so that great protraction of the infection resulted. With diamino-acridine sulphate, although a much larger dose was well tolerated (0·0015 grm.), there was, as a result, only very slight protraction of the infection as compared with the untreated controls. The latter invariably died, although the number of trypanosomes in the blood, after increasing to a maximum, frequently receded spontaneously, only to increase again prior to death. Thus, although the infection with this strain of trypanosomes in mice was by no means ideal for chemo-therapeutic observations, the fact of the great superiority of the acridinium compound in this respect was clearly apparent.

The main experiment consisted in introducing diamino-acridine sulphate in 0·85-per-cent. NaCl solution intravenously into rabbits, and then withdrawing specimens of blood at intervals. The blood was allowed to coagulate, and the serum was withdrawn under aseptic precautions, and was freed from cellular elements by centrifugalising. Quantities of 1 c.c. each of unheated serum were then inoculated with 0·1 c.c. of a 1 : 20,000 dilution of a 24-hour peptone water culture of *Staphylococcus aureus* or *B. coli*, and these cultures were incubated at 37° C. for 48 hours or longer. By way of control, a specimen of blood was taken before the injection, and was similarly inoculated. The majority of the animals manifested no signs of illness during or after the injections, and were alive and well many weeks later. The following are characteristic examples:—

Rabbit No. I (weight, 1950 grm.).—0·13 grm., dissolved in 45·5 c.c. of 0·85-per-cent. NaCl solution, injected into the auricular vein in the course of 9½ minutes (dose = 0·066 grm. per kilogramme of body weight).

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* We are indebted to Prof. Warrington Yorke for the strain.

A. Serum of blood taken before injection, inoculated with (*a*) *Staphylococcus aureus,* (*b*) *B. coli*; both gave abundant growth (marked turbidity after 24 hours at 37° C.).

B. Serum of blood withdrawn five minutes after the injection—

(i) Undiluted: remained perfectly clear after inoculation, in the case of both organisms, and subcultures on agar showed no growth;

(ii) A mixture of 50 per cent. serum B + 50 per cent. serum A gave no growth of either organism after inoculation;

(iii) A mixture of 25 per cent. serum B + 75 per cent. serum A yielded a growth of *Staphylococcus,* but not of *B. coli,* after inoculation.

Rabbit No. II (weight, 1850 grm.).—0·13 grm. dissolved in 40 c.c. of 0·85-per-cent. NaCl solution injected into the auricular vein in the course of 4½ minutes (dose = 0·07 grm. per kilogramme body weight).

A. Serum of blood taken before injection, inoculated with (*a*) *Staphylococcus aureus,* (*b*) *B. coli,* gave abundant growth after 24 and 48 hours at 37° C. respectively.

B. Serum of blood withdrawn 15–25 minutes after the injection—

(1) Undiluted: gave no growth after inoculation with either organism.

(2) Diluted with an equal volume of serum A: grew *Staphylococcus* but not *B. coli* after inoculation.

(3) Twenty-five per cent. serum B + 75 per cent. serum A gave a growth of *B. coli* after inoculation.

C. Serum of blood withdrawn 2½ hours after the injection—

(1) Undiluted: grew *Staphylococcus,* but not *B. coli* after inoculation.

(2) A 50 per cent. dilution with specimen A grew *B. coli* also.

Rabbit No. III (weight, 1420 grm.).—0·07 grm., dissolved in 20 c.c. of 0·85-per-cent. NaCl solution, injected into the auricular vein in the course of six minutes (dose = 0·05 grm. per kilogramme body weight).

A. Serum withdrawn before injection, inoculated with (*a*) *Staphylococcus,* (*b*) *B. coli,* gave abundant growths after 24 hours at 37° C. (turbid); the addition of 1 : 100,000 diamino-acridine sulphate to the serum *in vitro* prevented growth in the case of both organisms.

B. Serum withdrawn two hours after injection, when inoculated with *Staphylococcus aureus* and *B. coli,* remained perfectly clear after three days' incubation at 37° C., thus showing that little or no multiplication of the inoculated organisms had taken place, but subculture on agar yielded a few colonies in each case.

In the experiments quoted above fresh unheated serum was employed, and it might be inferred that the natural bactericidal property of serum, to which

Nuttall first drew attention, had contributed in a considerable measure to the results obtained; but it is to be noted that in all cases the controls, consisting of fresh serum obtained immediately before the injection, yielded after inoculation abundant growths of both organisms employed. Thus there is evidence in our experiments that the natural bactericidal property of the serum was not a decisive factor in producing the antiseptic effect. *Staphylococcus* is not killed by serum, as Wright and Windsor pointed out in the case of the human subject. In the case of *B. coli*, serum from the rabbit causes a phase of bactericidal action, which is frequently succeeded by multiplication (Chick); this latter phase of multiplication was of constant occurrence in our investigations.

In order to demonstrate further that the bactericidal effect following the injection of diamino-acridine sulphate was independent of properties of the fresh serum, experiments were also carried out with heated serum.

Example.—Rabbit (weight, 1510 grm.): 0·09 grm. diamino-acridine sulphate, dissolved in 20 c.c. of 0·85-per-cent. NaCl solution, injected into the auricular vein in the course of five minutes (dose = 0·06 grm. per kilogramme of body weight). Specimens of serum (*a*) fresh and (*b*) after heating for one hour at 56° C.—taken (A) before, (B) 17 minutes after, the injection—were inoculated with *Staphylococcus aureus* and *B. coli* as described above.

The results after incubation for 72 hours at 37° C. were as follows:—

Inoculation with *Staph. aureus.*

Serum.	A (before injection) undiluted	B (after injection) undiluted	B 75 per cent. + A 25 per cent.	B 25 per cent. + A 75 per cent.
Fresh	Abundant growth (turbid)	No growth* (clear)	No growth* (clear)	Growth (turbid)
Heated one hour at 56° C.	Abundant growth (turbid)	No growth* (clear)	No growth* (clear)	Growth (turbid)

Inoculation with *B. coli.*

Serum.	A undiluted	B undiluted	B 75 per cent. + A 25 per cent.	B 25 per cent. + A 75 per cent.
Fresh	Abundant growth† (turbid)	No growth* (clear)	No growth (clear)	No growth* (clear)
Heated one hour at 56° C.	Abundant growth† (turbid)	No growth* (clear)	No growth (clear)	Growth

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* Subcultures on agar yielded scanty colonies.

† With *B. coli* growth in the heated serum was abundant after 24 hours; in the unheated serum the growth was scanty after 24 hours, but became marked later; this may be taken as evidence of the natural inhibitory effect of fresh serum.

Thus, it has been shown that in rabbits, a dose of diamino-acridine sulphate, which is well tolerated when introduced directly in the blood stream, is capable of rendering the blood serum antiseptic or of augmenting greatly its bactericidal power, and that this property is still manifested several hours after the injection. A great part of the substance rapidly enters the muscles, which become of a distinct yellow tint, but this does not affect the fact just stated. Accordingly, there appears to be here a very promising indication as to the lines on which a chemo-therapeutic agent applicable to cases of bacterial septicæmia is to be sought.*

Diamino-acridine sulphate is absorbed from the alimentary tract, and, after administration by this route or intravenously, the urine soon exhibits the canary-yellow fluorescence, best seen on dilution, which is so characteristic of weak solutions of the acridine compounds. There is also excretion of the substance by the bile. Observations on the human subject treated by

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* We are indebted to Dr. H. H. Dale, F.R.S., for the blood-pressure record of an experiment in which he injected 0·3 grm. of the diamino-acridine sulphate in a volume of 100 c.c. intravenously into a monkey weighing 4·3 kgrm., under an anæsthetic. Specimens of serum taken during and after the injection (see below), both fresh, and also after heating for half an hour at 56° C., failed to yield growths after inoculation with staphylococcus and *B. coli.*

The results are as follows :—

Time.	Anæsthetic.	Blood-pressure at end of period.	Total amount of substance injected (1 : 300 solution) in each period.
Commencement of record	A.C.E.	100 mm. Hg	0
17 minutes later	„	90 „ „	0
16 „ „	„	75 „ „	22 c.c. run in intermittently.
18 „ „	„	Rapid fall towards the end of this period to 45 mm., and heart irregularly inhibited	40 c.c. run in continuously (at first 2 c.c. per minute, soon increased to 3 c.c. per minute)
15 „ „	Ether	55 mm. Hg	0 (20 c.c. blood withdrawn from carotid)
5 „ „	„	60 „ „	0
13 „ „	„	60 mm. Hg (heart rather severely inhibited)	38 c.c. (20 c.c. blood withdrawn from carotid)
21 „ „	„	60 mm. Hg (heart inhibition passed off)	0 (20 c.c. blood withdrawn, then animal bled completely)

The conclusion from this single experiment is that when an animal of similar susceptibility receives an intravenous injection under an anæsthetic, administration at as great a rate as 0·0025 grm. per minute per kilogramme of body weight causes some danger to the heart (under similar conditions this would mean that an average man weighing 60 kgrm. should not receive an intravenous injection at a rate exceeding 0·15 grm. per minute or 50 c.c. of a 1 : 330 solution). There is, of course, considerable likelihood that the anæsthetic tends to increase the susceptibility of the heart.

intravenous injections of diamino-methyl-acridinium chloride have shown that, after a dose of 0·15–0·3 grm. (in the form of a 1 : 1000 solution in physiological saline), fully a third of this amount can be accounted for in the urine passed during the subsequent two days.*

Thus it is possible that these or allied substances may prove of value in infections of the kidney and the biliary passages.

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* We are indebted to Captain T. F. Cotton, C.A.M.C., for the clinical material and to Dr. S. Russ for the estimations from absorption spectra. It is important that specimens of this substance to be employed for internal administration should be free from admixture with traces of poisonous metals.

The Isolation and Serological Differentiation of Bacillus tetani.

By W. J. Tulloch, M.D., Temp. Capt. R.A.M.C. (Lecturer on Bacteriology at the University of St. Andrews).

(Communicated by Sir David Bruce, F.R.S., Chairman of the W.O. Committee for the Study of Tetanus. Received November 9,—Revised December 28, 1917.)

(From the Laboratories of the Royal Army Medical College and the Lister Institute of Preventive Medicine.)

Synopsis.

As the history of bacteriology has shown that conditions regarded as clinical entities may be caused by a number of different organisms, it seemed of importance to determine whether the *Bacillus tetani* is an individual micro-organism, or whether there are several organisms differing from one another, but all capable of elaborating a spasm-producing toxin.

This enquiry involves the demarcation of the tetanus bacilli from similar organisms and their differentiation *inter se*, by serological methods.

By employing the agglutination reaction it can be shown that there are at least three serological types of *B. tetani*. One of these, which may be designated Type I, has hitherto been almost exclusively used for the preparation of antitoxin, and, so far as is known, such antitoxin protects against the toxin of all three types.

The results obtained in the present investigation suggest that this is only partially true, as the types of *B. tetani* isolated from cases of the disease do not correspond serologically with the type in common use for the preparation of antitoxin. This question is still under consideration, as the number of cultures so far examined is too limited to permit of far-reaching conclusions being drawn from the work done.

In attempting to isolate numbers of strains of *B. tetani* it is found that there are frequently present in the wounds of men suffering from tetanus, bacilli which have morphological characters so like those of *B. tetani* that they might readily be mistaken for that organism.

Serological investigation of these (non-toxic) micro-organisms, which might be referred to as tetanoid bacilli, shows that they too constitute a group, the members of which can be differentiated one from another by serological methods.

I. *Introduction.*

In view of the fact that bacteriological investigation has shown that conditions which had been regarded as clinical entities were really caused by infection with a number of different organisms, admittedly often closely allied to one another, but susceptible of classification by serological methods, it seemed of importance to determine whether, in the case of tetanus, we are dealing with an infection due to one and the same organism in all cases of the disease, or to a number of different though closely related bacteria.

The differentiation of the dysentery bacilli, of the enteric group of organisms, of the pneumococci, and of the meningococci, indicated that examination should be made of the causal organism of tetanus by all bacteriological, and especially by serological methods.

II. *Cultivation of* B. tetani.

The main obstacle to be overcome in studying *B. tetani* is the difficulty experienced in isolating the organism. Owing to its living in symbiosis with other bacteria that grow more vigorously in artificial media than does *B. tetani,* special methods have had to be elaborated for the (partial) isolation of that organism.

The *rationale* of the technique which I use depends upon the fact that if material from wounds infected with the anaërobes be inoculated into a medium consisting of chopped meat and water, sterilised in the autoclave, the proteolytic organisms—notably *B. sporogenes*—first appear in the culture and are replaced later by organisms which resemble the tetanus bacillus, both morphologically and, to some extent, culturally. Many of these organisms are non-pathogenic, and from the standpoint of cultural requirements *B. tetani* is included with them.

After several attempts with a variety of procedures, I have so far found the following method most satisfactory for my purpose :—

Take 1 lb. of chopped meat and add 1 litre of tap-water, then boil for 30 minutes. Cool to 45° C., make slightly alkaline, and add trypsin as for the preparation of Douglas' broth, then incubate in an open vessel at 37° C. for five days, and allow to undergo natural putrefaction.

The putrescent material so obtained is filtered through paper, made neutral to phenolphthalein, and sodium formate is added to the extent of 1 per cent. of the total. The material is then sterilised and cleared by being passed through a Berkfeld and a Doulton filter in series. The medium is stored in sterile flasks under paraffin, and is syphoned off as required. It keeps well, but should not be sterilised by heat.

Before use the sterility of the medium is tested by inoculating quantities of from 5 to 0·1 c.c. into tubes of meat-water medium, which are incubated anaërobically for seven days. This medium, while it has desirable selective properties, is not sufficiently nutrient, and must be enriched before use by the addition of fresh rabbit tissue. Prepared in this way, the medium inhibits the growth of *B. sporogenes,* and allows of the growth of *B. tetani,* atoxic tetanoid bacilli, and oval-endsporing organisms. The value of this medium, in its application to the study of *B. tetani,* will be considered later.

III. *Application of Serological Methods to the Study of Toxic Cultures.*

Using this putrid medium and Barber's micro-inoculation method, I succeeded, with the assistance of Miss Robertson, of the Lister Institute of Preventive Medicine, in isolating from wounds a small number of strains of *B. tetani* in a fair state of purity.

Particular attention is called to the question of purity of cultures of the anaërobes. I advisedly describe the growths which are under consideration as being only relatively pure, since extreme difficulty is experienced in completely purifying these cultures. This difficulty is due probably to the symbiotic relationship which the anaërobes bear to one another.

I would here record my deep debt of gratitude to Miss Robertson for her kindness in purifying these growths for me, as they formed the basis for serological methods being applied to the examination of the question under consideration.

Having obtained from wounds a number of fairly pure growths known to be toxic, I immunised a rabbit against one of the laboratory strains of *B. tetani,* with a view to the production of an agglutinating serum.

The strain of *B. tetani* chosen was an isolation made by Miss Robertson from the U.S.A. standard culture. This culture will hereafter be designated "A." The process of immunising the animals was as follows:—

A culture of the organism to be inoculated was grown in peptone broth neutral to α-naphtholphthalein under anaërobic conditions at 37° C. for a period of four days. The cultures were then heated to 60° C. for 30 minutes, and thereafter centrifugalised at high speed to deposit the organisms. The supernatant fluid was pipetted off, saline added, and the suspension so obtained was again centrifugalised.

The deposit resulting from this second centrifugalisation was shaken in a small quantity of saline, and the resulting suspension was standardised. Sufficient saline, containing 0·5 per cent. phenol, was then added to reduce the bacillary content of the suspension to 2000 million per cubic centimetre.

In immunising the animals, 0·75 c.c. of this suspension is inoculated intravenously, and, after an interval of five days, 1·5 c.c. is injected by the same route; after a further lapse of two days, the agglutinating titre of the serum is tested, and, if suitable, the animal is killed. No difficulty was experienced in obtaining a serum of moderate titre—1/800 in two hours at

55° C. was aimed at—and, using this serum to agglutinate laboratory strains and strains isolated from wounds, the following results were obtained:—

Diagram I.—Agglutination of Toxic Cultures with Serum of Animal Immunised with Culture A.

Designation of culture.	Source whence culture was obtained.	Dilutions of serum.			Normal serum.
		1/100.	1/200.	1/400.	1/50.
A	Isolation from U.S.A. culture ...	+ +	+ +	+ +	–
B	,, ,, ,, ...	+ +	+ +	+ +	–
C	Whole culture, U.S.A. standard	+ +	+ +	+ +	–
D	Serum Institute X	+ +	+ +	+ +	–
E	,, ,, X	+	+	+	–
F	,, ,, Y	+ +	+ +	+ +	–
G	,, ,, Y	+ +	+ +	+ +	–
H	,, ,, Z	+ +	+ +	+ +	–
1	Isolated from wound	+ +	+ +	+ +	–
2	,, ,, ...	–	–	–	–
3	,, ,, ...	–	–	–	–
4	,, ,, ...	–	–	–	–
5	,, ,, ...	–	–	–	–
6	,, ,, ...	–	–	–	–
Control	Bacillary suspension used for immunisation.	+ +	+ +	+ +	–

Readings after 2 hours at 55° C.

+ + = complete flocculation, the supernatant fluid being clear.
+ = agglutination obvious to the naked eye.
(+) = agglutination only in comparison with control tube of emulsion + 1/50 normal serum.

It will be seen that only one of the toxic cultures—1—derived from a wound agglutinates with this serum, while, on the contrary, all the laboratory strains give a positive reaction.

Culture 2 was therefore used for immunising a second animal, which was bled out on attaining a titre of 1/800 when tested in presence of its homologous organism.

Using this serum, the results tabulated in Diagram II were obtained.

It is therefore found that three of the organisms which failed to react in presence of the agglutinating serum from the U.S.A. cultures are flocculated by this serum.

Culture 6 was used for immunising a third animal, and with this serum the remaining bacilli 6 and 3 gave a positive reaction.

Diagram II.—Agglutination of Toxic Cultures with Serum of Animal Immunised with Culture 2.

Designation of culture.	Source whence culture was obtained.	Dilutions of serum.			Normal serum.
		1/100.	1/200.	1/400.	1/50.
A	Isolation from U.S.A. culture ...	—	—	—	—
B	„ „ „ ...	—	—	—	—
C	Whole culture, U.S.A. standard	—	—	—	—
D	Serum Institute X	—	—	—	—
E	„ „ X	—	—	—	—
F	„ „ Y	—	—	—	—
G	„ „ Y	—	—	—	—
H	„ „ Z	—	—	—	—
1	Isolated from wound	—	—	—	—
2	„ „	+ +	+ +	+ +	—
3	„ „	—	—	—	—
4	„ „	+ +	+ +	+ +	—
5	„ „	+	+	—	—
6	„ „	—	—	—	—
Control	Bacillary suspension used for immunisation	+ +	+ +	+ +	—

Readings after 2 hours at 55° C.

Diagram III.—Agglutination of Toxic Cultures with Serum of Animal Immunised with Culture 6.

Designation of culture.	Source whence culture was obtained.	Dilutions of serum.			Normal serum.
		1/100.	1/200.	1/400.	1/50.
A	Isolation from U.S.A. culture ...	—	—	—	—
B	„ „ „ ...	—	—	—	—
C	Whole culture, U.S.A. standard	—	—	—	—
D	Serum Institute X	—	—	—	—
E	„ „ X	—	—	—	—
F	„ „ Y	—	—	—	—
G	„ „ Y	—	—	—	—
H	„ „ Z	—	—	—	—
1	Isolated from wound	—	—	—	—
2	„ „	—	—	—	—
3	„ „	+ +	+ +	+ +	—
4	„ „	—	—	—	—
5	„ „	—	—	—	—
6	„ „	+ +	+ +	+ +	—
Control	Bacillary suspension used for immunisation	+ +	+ +	+ +	—

Readings after 2 hours at 55° C.

It appears, then, that there are at least three serological types of organisms capable of producing a tetanising poison, but it might be justifiably argued that the agglutination of Cultures 2, 4, and 5, and of 3 and 6, was due to the presence of a common contaminating organism in each instance.

To exclude such possible error, the following strains of organisms which were known to be non-toxic, and which superficially resembled *B. tetani,* both culturally and in morphological characters, were exposed to three "type sera," corresponding to the three serological varieties that the foregoing experiments demonstrate, and also to sera which agglutinated *B. sporogenes, B. pseudo-tetani,* and an organism superficially resembling *B. tetani,* but known to be non-toxic. This non-toxic organism and others similar to it are frequently present in wounds infected with *B. tetani.* I shall refer to it as "tetanoid bacillus No. I," as there appears to be a group of such organisms which can be differentiated from *B. pseudo-tetani, B. Hibler IX,* and from one another by serological methods. Diagram IV indicates the results obtained on exposing these non-toxic bacilli to the action of tetanus and other agglutinating sera.

It is seen from this result and from Diagram I that Culture E is a mixed growth of two organisms:

(1) Corresponding to Tetani U.S.A. (Type I).
(2) „ „ Tetanoid No. 1.

In the succeeding sections of the present communication those (toxic) bacilli which give a positive agglutination in presence of the serum of an animal immunised against Culture A (U.S.A. bacillus) will be referred to as tetanus bacilli of No. I serological type, those which react in the same way as Culture 2 as of the No. II serological type, and those which behave as does Culture 6 as No. III serological type.

IV. *Examination of Organisms resembling* B. tetani *obtained by Direct Cultivation of Wound Exudates in the Putrescent Medium.*

In order further to test the validity of the deductions made from the result obtained on examining the non-toxic organisms, and to determine, if possible, the frequency with which the various types of *B. tetani* are found in wound exudates, I carried out the following experiment.

Cultures from wound exudates in cases of tetanus, or from old meat cultures, were made, (1) directly into the selective medium, and (2) the same inocula were grown in broth under anaërobic conditions for eight days.

The growths in the selective medium were filtered through a loose plug of cotton-wool to remove detritus, centrifuged, and the deposit suspended in

Diagram IV.

Designation of culture.	Agglutinating sera for toxic bacilli.						Agglutinating sera for other organisms.						Normal serum.
	Tetanus A.		Tetanus 2.		Tetanus 6.		Pseudo-tetani.		Sporogenes.		Tetanoid No. 1.		
	1/100.	1/200.	1/100.	1/200.	1/100.	1/200.	1/100.	1/200.	1/100.	1/200.	1/50.	1/100.	1/50.
a	—	—	—	—	—	—	—	—	—	—	—	—	—
b	—	—	—	—	—	—	—	—	—	—	—	—	—
c	—	—	—	—	—	—	—	—	—	—	+ +	+ +	—
d	—	—	—	—	—	—	—	—	—	—	+ +	+ +	—
e	—	—	—	—	—	—	—	—	—	—	+ +	+ +	—
f	—	—	—	—	—	—	—	—	—	—	+ +	+ +	—
g	—	—	—	—	—	—	—	—	—	—	—	—	—
h	—	—	—	—	—	—	—	—	—	—	+	+	—
i	—	—	—	—	—	—	—	—	—	—	—	—	—
j	—	—	—	—	—	—	—	—	—	—	—	—	—
Controls—													
Tetanus A	+ +	+ +	—	—	—	—	—	—	—	—	—	—	—
„ 2	—	—	+ +	+ +	—	—	—	—	—	—	—	—	—
„ 6	—	—	—	—	+ +	+ +	—	—	—	—	—	—	—
Pseudo-tetani	—	—	—	—	—	—	+ +	+ +	—	—	—	—	—
Sporogenes	—	—	—	—	—	—	—	—	+ +	+ +	—	—	—
Tetanoid No. 1	—	—	—	—	—	—	—	—	—	—	+ +	+ +	—

Readings taken after 2 hours at 55° C.

Note.—Culture *c* in the above diagram is the same organism as that used for immunising animal to obtain Tetanoid No. 1 agglutinating serum.

Diagram IVA.—Influence of Agglutinating Sera Specific to Non-Toxic Organisms upon the Toxin Producing Tetanus Bacilli whose Reactions are noted in Diagrams I, II, and III.

Designation of culture.	Whence culture was obtained.	Sporogenes—Agglutinating serum.			Pseudo-tetani—Agglutinating serum.			Tetanoid No. 1—Agglutinating serum.			Normal serum.
		1/100.	1/200.	1/400.	1/100.	1/200.	1/400.	1/50.	1/100.	1/200.	1/50.
A	*Vide* Diagram I	–	–	–	–	–	–	–	–	–	–
B	,, ,,	–	–	–	–	–	–	–	–	–	–
C	,, ,,	–	–	–	–	–	–	–	–	–	–
D	,, ,,	–	–	–	–	–	–	–	–	–	–
E	,, ,,	–	–	–	–	–	–	+ +	+	+	–
F	,, ,,	–	–	–	–	–	–	–	–	–	–
G	,, ,,	–	–	–	–	–	–	–	–	–	–
H	,, ,,	–	–	–	–	–	–	–	–	–	–
1	Wound	–	–	–	–	–	–	–	–	–	–
2	,,	–	–	–	–	–	–	–	–	–	–
3	,,	–	–	–	–	–	–	–	–	–	–
4	,,	–	–	–	–	–	–	–	–	–	–
5	,,	–	–	–	–	–	–	–	–	–	–
6	,,	–	–	–	–	–	–	–	–	–	–
Sporogenes	,,	+ +	+ +	+ +	–	–	–	–	–	–	–
Pseudo-tetani	,,	–	–	–	+ +	+ +	+ +	–	–	–	–
Tetanoid No. 1	,,	–	–	–	–	–	–	+ +	+ +	+	–

Readings taken after two hours at 55° C.

saline. The deposit was examined microscopically to determine the presence of endsporing bacilli in overwhelming numbers.

The saline suspension was standardised by the opacity method and exposed to the action of agglutinating sera specific to the three toxic types, and also to sera specific to *B. sporogenes*, *B. pseudo-tetani*, and Tetanoid No. 1 bacillus.

The eight-day broth culture was inoculated into animals in a dose of 0·5 c.c.

Owing to the difficulty of obtaining guinea-pigs for this purpose I was compelled to employ rats. The animals were inoculated subcutaneously at the root of the tail. Diagram V (p. 154) illustrates the results obtained.

The points calling for comment in these results are:—

(*a*) The agglutination and toxicity tests agree in all but two cultures—84 and 89.

Whether these two cultures represent a fourth type of the bacillus or not I cannot at present state definitely, but from the late development of symptoms of tetanus in the animals inoculated with the broth cultures corresponding to these, it appears to me to be probable that the inocula contained but few *B. tetani* and the agglutination was masked by the overplus of other organisms present in the suspension.

(*b*) Up to the present the type of *B. tetani* most frequently obtained from wound exudates in cases of tetanus among men who have received prophylactic inoculation, corresponds to the culture designated Type II. The number of cultures examined is at present too small to permit of any far-reaching conclusion being drawn from the results obtained.

The following agglutination results with four toxic cultures are of peculiar interest, in that these organisms were derived from wounds in men not suffering from tetanus.

Diagram V.

Designation of culture.	Growth to be agglutinated was grown in the selective medium from :—	Tetanus agglutininating.			Non-toxic agglutinating serum.			Normal Serum.	Result of animal test for toxicity.
		Type I.	Type II.	Type III.	Pseudo T.	Sporogenes.	Tetanoid.		
		1/100.	1/100.	1/100.	1/100.	1/100.	1/50.	1/50.	
3	From old culture in meat	+ +	–	–	–	–	–	–	Spasm in 24 hours.
28	” ” ”	–	–	–	–	–	–	–	Animal remained healthy.
32	” ” ”	–	–	–	–	–	–	–	” ” ”
47	” ” ”	–	–	–	–	–	–	–	” ” ”
65	” ” ”	–	–	–	–	–	+ +	–	” ” ”
77	” ” ”	–	–	–	–	–	–	–	” ” ”
81	Cultivated directly from wound	–	+ +	–	–	–	–	–	Died within 24 hours.
83	” ” ”	–	+ +	–	–	–	–	–	” ”
84	” ” ”	–	–	–	–	–	–	–	Local tetanus in 48 hours.
85	” ” ”	–	–	+ +	–	–	–	–	Died within 24 hours.
86	” ” ”	–	–	–	–	–	–	–	Animal remained healthy.
87	” ” ”	–	–	–	–	–	+ +	–	” ” ”
88	” ” ”	–	+ +	–	–	–	+ +	–	Died within 24 hours.
89	” ” ”	–	–	–	–	–	–	–	Local tetanus 4th day.
90	” ” ”	–	+ +	–	–	–	–	–	Died within 24 hours.
91	” ” ”	–	–	–	–	–	–	–	Animal remained healthy.
Controls	——	+ +	+ +	+ +	+ +	+ +	+ +	–	

Readings taken after 2 hours at 55° C.

Diagram VI.

Designation of culture.	Culture to be agglutinated was grown in the selective medium from :—	Tetanus agglutinating serum.			Non-toxic agglutinating serum.			Normal serum.
		Type I.	Type II.	Type III.	Pseudo T.	Sporogenes.	Tetanoid.	
		1/100.	1/100.	1/100.	1/100.	1/100.	1/50.	1/50.
R 27	Broth culture known to be toxic.	+ +	—	—	—	—	—	—
R 62	,, ,,	+	—	—	—	—	—	—
R 68	,, ,,	+ +	—	—	—	—	—	—
R 146	,, ,,	—	+ +	—	—	—	—	—
Controls	——	+ +	+ +	+ +	+ +	+ +	+ +	—

Readings taken after 2 hours at 55° C.

It is interesting to note that the non-toxic culture Tetanoid No. 1 was isolated from the whole culture R 27, which as is seen from the above diagram is a toxic tetanus bacillus of the U.S.A. type (Type I). The culture R 27 was obtained from a man 49 days after the infliction of his wound. He had received one dose of tetanus antitoxin on the day of wounding. Cultures R 62 and R 68, which are also of the U.S.A. type (Type I), were both obtained on the 56th day after the infliction of the wound. In the case of one of these, the man is not clear whether he had or had not received a dose of antitoxin at the time of wounding. Culture 146 was obtained on the 8th day after wounding. A dose of antitoxin had been administered in this case. Re-examination of the wound made 30 days after the infliction of the wound showed that no anaërobic organisms of any kind whatsoever were present in the exudate.

It is significant that three of these four toxic cultures, obtained from men not showing evidence of tetanus intoxication, are of the U.S.A. type, particularly in view of the fact that of the cultures obtained up to the present from cases showing symptoms of tetanus occurring for the most part in inoculated men, only one culture of the U.S.A. serological type has been obtained. In my opinion this may mean that the prophylactic inoculation of the antitoxin at present in use protects strongly against intoxication from the U.S.A. type of bacillus, while its protection against the other types is apparently short lived.

V. *Absorption of Agglutinin Tests.*

In order to be satisfied that the agglutination results, tabulated in Diagrams I, II, and III, indicated definite antigenic differences between the three types of bacilli, I carried out a number of absorption of agglutinin tests.

Two representatives of each serological type of the bacillus were chosen for this test:—

Representatives of Type I:—

(i) A, an isolation from the U.S.A. culture.
(ii) C, the standard U.S.A. culture.

Representatives of Type II:—

(i) 2, isolated from a wound.
(ii) 5, ,, ,, ,, (this culture was selected because it had failed to agglutinate to the full titre in presence of Type II serum).

Representatives of Type III:—

(i) 3, isolated from a wound.
(ii) 6, ,, ,, ,,

Each serum was absorbed by contact with suspensions of each of these cultures thus:—

Three horizontal rows consisting of six centrifuge tubes were set up. Into each tube of the first row was pipetted 0·05 c.c. of Type I serum; into those of the second row 0·05 c.c. of Type II serum, and of the third, Type III serum.

There was then added to each tube in the vertical columns (consisting of three tubes each) 2·5 c.c. of a 2,000,000,000 suspension of bacilli,

(*a*) to those of column 1, Culture A.
(*b*) ,, ,, 2, ,, C.
(*c*) ,, ,, 3, ,, 2.
(*d*) ,, ,, 4, ,, 5.
(*e*) ,, ,, 5, ,, 3.
(*f*) ,, ,, 6, ,, 6.

The tubes were incubated at 37° C. for 24 hours, centrifuged, and the clear supernatant fluid from each was then distributed into agglutination tubes in such dilution that with the addition of bacillary suspension the concentration of the sera in each series would be 1/100, 1/200, 1/400.

The absorbed serum in each case was used for agglutinating suspensions both of the homologous bacillus and the test bacillus.

PROCEEDINGS
OF
THE ROYAL SOCIETY.

Series B. Vol. 90. **No. B 627.**

BIOLOGICAL SCIENCES.

CONTENTS.

Page

The Formation of Nitrites from Nitrates in Aqueous Solution by the Action of Sunlight, and the Assimilation of the Nitrites by Green Leaves in Sunlight. By BENJAMIN MOORE, D.Sc., F.R.S. 158

Action of Light Rays on Organic Compounds, and the Photo-synthesis of Organic from Inorganic Compounds in Presence of Inorganic Colloids. By BENJAMIN MOORE, D.Sc., F.R.S., and T. A. WEBSTER . . . 168

Growth of Trees, with a Note on Interference Bands formed by Rays at Small Angles. By A. MALLOCK, F.R.S. 186

On the Efficiency of Muscular Work. By M. GREENWOOD, Captain R.A.M.C. (T.F.), Lister Institute of Preventive Medicine . . . 199

The Galvanometric Measurement of "Emotive" Physiological Changes. By A. D. WALLER, M.D., F.R.S. 214

Obituary Notice of Fellow deceased :—Edward Hull (with portrait) xxviii—xxxi

PRINTED FOR THE ROYAL SOCIETY AND SOLD BY
HARRISON & SONS, 45, ST. MARTIN'S LANE, LONDON, W.C.

Price Three Shillings and Sixpence.

No. B 627.

June 1, 1918.

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The serum was considered to be absorbed if its titre was reduced to not more than 1/100, from 1/400.

The results were controlled by reactions with unabsorbed serum in the same series of dilutions 1/100, 1/200, 1/400.

As the following Table shows, the absorption of agglutinin test confirms the previous observations and shows that considered as antigenics, the three types are distinct from one another.

Diagram VII.—Agglutinating Serum.

Designation of culture.	Unabsorbed serum—Test bacillus added.			Absorbed serum—Homologous bacillus added.			Absorbed serum—Test bacillus added.		
	1/100.	1/200.	1/400.	1/100.	1/200.	1/400.	1/100.	1/200.	1/400.
Type I.									
A (Type I)	+ +	+ +	+ +	–	–	–	–	–	–
C "	+ +	+	+	(+)	–	–	–	–	–
2 (Type II)	–	–	–	+ +	+ +	+ +	–	–	–
5 "	–	–	–	+ +	+ +	+ +	–	–	–
3 (Type III)	–	–	–	+ +	+ +	+ +	–	–	–
6 "	–	–	–	+ +	+ +	+ +	–	–	–
Type II.									
A	–	–	–	+ +	+ +	+ +	–	–	–
C	–	–	–	+ +	+ +	+ +	–	–	–
2	+ +	+ +	+ +	(+)	–	–	(+)	–	–
5	+ +	+ +	+	–	–	–	–	–	–
3	–	–	–	+ +	+ +	+	–	–	–
6	–	–	–	+ +	+ +	+ +	–	–	–
Type III.									
A	–	–	–	+ +	+ +	+ +	–	–	–
C	–	–	–	+ +	+ +	+	–	–	–
2	–	–	–	+ +	+ +	+ +	–	–	–
5	–	–	–	+ +	+ +	+	–	–	–
3	+ +	+ +	+	–	–	–	–	–	–
6	+ +	+ +	+ +	–	–	–	–	–	–

VI. *Are the Toxins Produced by each Type in any way Specific, showing Differences Corresponding to those made Manifest by the Agglutination Reaction in the Case of the Bacilli?*

This question has not yet been gone into, but will form the subject of further research, for the determination of such a specificity of toxin is of paramount import in view of the influence it would have upon the prophylaxis and therapeutics of the disease.

Conclusions.

The following conclusions may, I think, be justifiably drawn from these observations.

(1) More than one variety of (non-toxic) endsporing bacillus, resembling *B. tetani* in morphological characters, can be recovered from wound exudates in cases of the disease.

(2) There are at least three different types of (toxic) *B. tetani.*

(3) The "U.S.A. type" of the bacillus—that commonly used for the preparation of antitoxin—is not frequently obtained from wound exudates in cases of the disease occurring among men who have received prophylactic inoculations of antitetanic serum.

(4) Culture in a selective medium, followed by agglutination of the washed growth in presence of the three type sera, gives valuable information. It is, however, apparently not so delicate a test for the presence of *B. tetani* as is animal inoculation after culture of the wound exudate.

The Formation of Nitrites from Nitrates in Aqueous Solution by the Action of Sunlight, and the Assimilation of the Nitrites by Green Leaves in Sunlight.

By BENJAMIN MOORE, D.Sc., F.R.S.

(Received October 12, 1917.)

(From the Department of Applied Physiology and Hygiene of the Medical Research Committee.)

The number of chemical changes brought about by the activity of light is multitudinous, and the study of these reactions has been very intensive in recent years. In the majority of the photo-chemical reactions, the effect produced is that of hastening an exothermic reaction, and in this resembles the action of a catalyst. The substances formed have a less content of chemical energy than the mother substances, and are usually of a more simple structural type. In such cases there is no clear proof of transformation, or conversion, of light-energy into chemical energy, and the light acts more as a detonator to a chemical reaction in which chemical energy is set free.

The most important case of an endothermic reaction set up by the action of light is that in which the synthesis of formaldehyde and carbohydrate is effected in the green leaf, by that action of light upon water and carbon dioxide in which the light-energy is converted into chemical energy and stored up.

It has been shown by Moore and Webster (1) that the first step, namely, that of formaldehyde formation, in which the greatest upbuilding of molecules with large storage of chemical energy occurs, can be effected by certain catalysts, such as the ferric and uranium salts in colloidal solution in water, when these are supplied with light-energy. Later, it was shown by Moore (2) that such inorganic catalysts are present in the chloroplasts of green cells.

If it be agreed that life at some period first arose on this or some other planet by a process of evolution from simpler constituents, it is clear that there must have arisen along the path of evolution some combination or mechanism for forming more complex molecules containing the elements carbon, hydrogen, oxygen and nitrogen, from simpler inorganic substances with a less content of energy. Otherwise, the substratum from which the living organism was to be built could not exist, and there would have been no store of existent chemical energy to act as nutriment for the simplest living organism and supply the essential energy for the acts of life.

The assumption that the green cell, with its complex structure and exceedingly highly organised chemical substances, such as the chlorophylls, formed at the dawn of life the first engine capable of utilising light-energy and producing a gain of chemical energy is entirely untenable, and would make a break in the continuity of inorganic and organic evolution, such as nowhere else is to be found.

It hence becomes of great importance to study fully the action of light upon those inorganic substances which are present in air and water, and might be presumed, from their nature and present position as nutrients of living organisms, to have been capable of being acted upon by light with inductance of endothermic chemical reactions, and formation of more complex compounds of organic character.

Our knowledge of the first steps in the assimilation of both carbon and nitrogen so as to take their part in the organic compounds is still very incomplete, but that regarding nitrogen assimilation is much the more fragmentary of the two.

It is true that the living cell can by linkage of reactions utilise the energy stored up in the form of carbohydrates, to induce endothermic reactions and build up fats and proteins. As has been pointed out by Moore (3), a certain amount of carbohydrates can be oxidised, and the energy so set free within the cell can be transferred to reduce another portion of carbohydrate to fat, or to reduce nitrogenous compounds and build in amino-groups to form proteins. Other examples of such linked reactions are seen in the action of certain bacteria and other unicellular organisms, such as Azotobacter, the nitro-bacteria of the nodules of the Leguminosæ, the philothionic organisms which derive stores of energy from the oxidation of sulphur or reduced sulphur compounds, and the iron bacteria which similarly utilise the energy obtained by oxidation of metallic iron or of ferrous compounds to build up organic carbon compounds from carbon dioxide and water. Such linked reactions require, however, the presence of a living cell containing protoplasm, possessing as its substratum organic compounds containing both carbon and nitrogen in very complex combinations. Moreover, the substrata of reduced compounds so utilised in linked reactions have demanded at earlier epochs the existence of living organisms for their reduction by the conversion of the energy of sunlight. None of these substances could have existed in a planet cooling down from a red-hot condition, on account of their chemical instability at higher temperatures. So also all the bound nitrogen in vegetable and animal organisms, and their decomposition products, such as coal, guano, and nitrates, must at one time have existed as atmospheric nitrogen, for no nitrates or nitro-compounds could have withstood the earlier high temperatures. The enormous stores of compounds containing the oxides of nitrogen now used in warfare, agriculture, and industry must have been formed endothermically from atmospheric nitrogen and oxygen with uptake of energy, and whether this occurred through the electric discharge of the thunderstorm or by the agency of living organisms, the first source of the energy, just as in the case of the organic carbon compounds, was the sun's rays. It follows that the agencies by which sunlight was utilised to form reduced compounds of carbon and nitrogen must have existed antecedently to the advent of life, for in its ultimate composition the substratum of proteins necessary to the living organism contains both types of endothermically produced radicles. It was such considerations which induced the series of experiments here recorded, which show that the energy of sunlight can be absorbed by dilute solutions of nitrates and institute an endothermic reaction in which the more reactive nitrites are formed even in absence of living organisms, and also that the green cells of plants possess the power of absorbing these nitrites.

It is well known from the thermo-chemical determinations of Faure, Thomsen, and Berthelot that in the formation of the oxides of nitrogen from their elements the acme of absorption of energy lies at the point of formation of nitric oxide (N_2O_2), and that the reaction runs endothermically towards this point, whether the starting point be nitrous oxide (N_2O) or nitrogen pentoxide (N_2O_5). The amounts of heat involved and differences in transition from one oxide to another are shown succinctly in the following Table abstracted from Mendeléeff (4), in which the numbers in the upper row represent thousands of gramme-calories for a gramme-molecular formation from the elements; and the lower shows in thousands of gramme-calories the heats of transition from one oxide to the other:—

$$\begin{array}{ccccccccc} N_2O & & N_2O_2 & & N_2O_3 & & N_2O_4 & & N_2O_5 \\ -21 & & -43 & & -22 & & -5 & & -1 \\ & -22 & & +21 & & +17 & & +4 & \\ & & & & \longrightarrow & & & & \end{array}$$

This Table shows that in passage from N_2O_5 to N_2O_3, a supply of energy must be given to the reacting system amounting to $4+17 = 21$ thousands of gramme-calories for each gramme-molecule converted. The figures are for the gaseous condition, but it may be taken that they would be approximately the same for dilute solutions, and so that the amount of energy for the passage of a gramme molecule of a nitrate to a nitrite (say, from KNO_3 to KNO_2) would be about half this amount or about 10,000 grm. cal., for 1 grm. mol. of nitrogen pentoxide yields two of nitrate, which is converted into two of nitrite. These figures show clearly that the transition from nitrate to nitrite is a strongly endothermic reaction, and can only occur either by transformation of other forms of energy, such as that of light, into chemical energy, or by a linked chemical reaction with oxidation of previously formed reduced chemical substances.

Not only do the nitrites contain a greater storage of chemical energy than the nitrates, the energy potential factor of the energy quantity possesses a higher value, so that the nitrites react more readily than the nitrates, and many changes occur between living organisms and nitrites which are not given directly by nitrates. The experiments recorded below show that this endothermic reaction occurs in sunlight when dilute solutions of nitrates are exposed to the direct rays of the sun, either dissolved in redistilled water or as they occur in natural waters. In addition, it has been found that green leaves immersed in water possess, in presence of sunlight, the power of absorbing the nitrites so formed in the water.

In the earlier stages of the investigation the source of the nitrites obtained was not clearly understood. The outset point was that the nitrites and nitrates found to be present in atmospheric air by many previous observers could not be satisfactorily explained on the basis of the disruptive electrical discharges of thunderstorms, because there has not been traced any definite correspondence between the amount of nitrites and nitrates in air and rain-water and the prevalence of thunderstorms, such as must undoubtedly exist, were the energy of the lightning discharge the main cause of the production of nitrites and nitrates in the air.

Rain collected in the course of the present experiments when there had been no recent thunder was found to contain nearly as much nitrite (about 0·5 part per million) as rain caught in a thunderstorm, and, as has been shown by Ilosvay (5), the morning dew contains nitrites. Also, although the amounts of nitrites and nitrates present at any given time in the air are small, the amounts abstracted by condensing aqueous vapour and falling as rain or condensing as dew on the surfaces of leaves and ground in the course of the year is enormous, and this would appear to demand some uniformly distributed and more constantly acting source of energy, such as sunlight, rather than be dependent upon fortuitous electrical discharges.

These nitrites of the rain and dew form one of the chief supplies of nitrogenous nutrition for plants and animals supported by soil not artificially enriched with nitrogenous manure; the experiments given below indicate that there is also a probable aërial uptake of nitrites by the green leaves.

A source of much error and confusion in estimating the so-called "active" oxygen of air, rain, or dew by different observers at meteorological stations has been the use of test-papers, impregnated with starch and iodides, which were moistened and exposed to air and indicated, by the rapidity of development of a blue colour, the degree of "active" oxygen in air. This "active" oxygen was assumed to be present mainly as ozone or hydrogen peroxide without more proof, but this liberation of iodine from iodides is accomplished quite as readily by nitrogen tri- or tetr-oxide as by ozone or hydrogen peroxide. All the more recent researches (7) indicate that ozone and hydrogen peroxide are absent from the air at the earth's surface, and the important purifying and bleaching reactions hitherto ascribed to them must now be transferred to the intermediate oxides of nitrogen. At high dilutions, such as are shown below to occur under atmospheric conditions, the odours of ozone and that of the oxides of nitrogen are indistinguishable.

The present experiments show that air, rain, and dew invariably contain a mixture of nitrites and nitrates, and that on keeping the nitrites pass over into nitrates, but by insolation this process is reversed, and nitrites are formed from nitrates.

The test used was Ilosvay's modification of the diazo-reactions discovered by Griess, yielding compounds deeply coloured even at high dilutions; one of the best of these reactions for the purpose is that in which solutions of sulphanilic acid and α-naphthylamine in acetic acid are added to the water suspected of containing nitrites (8). This test is given only by nitrites, and not by either ozone or hydrogen peroxide.

Ilosvay (6) by the use of this test showed that the well-known reaction upon a paper impregnated with starch and potassium iodide often used to show the supposed presence of ozone in the atmosphere was really produced by nitrites, and demonstrated that at the earth's surface both ozone and hydrogen peroxide were normally absent. The same observer (5) found a strong reaction for nitrites in the morning dew on various leaves and grasses, and also adsorbed upon ignited sand and upon earth exposed wet to the atmosphere, and in water in absorption tubes through which air was drawn.

This test will clearly indicate, by the development of a pink colour, the presence of nitrites in a dilution of one in ten million. The test, when applied to the solutions exposed to sunlight, as described below, gave reactions indicating amounts of nitrite lying between two in a million and one in ten million.

These concentrations may appear at first sight infinitesimally low, but attention must be paid to the enormous areas in green leaves over the earth's surface which are exposed to the reaction. The strengths of solutions from which living organisms absorb essential constituents from their environments often belong to this order of concentration. The concentration of silicic acid in pond water, from which diatoms build up their siliceous skeletons, is of the same order of magnitude. A similar condition of affairs emerges if the assimilation of carbon compounds is considered, for all such assimilation depends on a concentration of only about three parts *by volume* of carbon dioxide in 10,000 of atmospheric air.

The concentration of carbon dioxide in the atmosphere of 3 parts in 10,000 by volume, small as it may appear to support all life upon the earth, looks at first sight enormous, compared to the concentrations at which silica is absorbed in plants, or to the concentrations of nitrites with which we are dealing in the present experiments; but this arises entirely from the usual conventional mode of expression of the concentration in relative gaseous volumes in the atmosphere.

If the mass of carbon dioxide dissolved in water be expressed in relationship to the mass of water, the ratio drops to the same order of magnitude as obtains in the case of other essential constituents demanded for organic life, all of which, it must be remembered, including carbon, are synthesised from solutions and not in gaseous form. Thus, the absorption-coefficient of carbon dioxide between a system of air and water may be taken sufficiently accurately for these purposes as equivalent to unity, so that if an atmosphere containing 3 parts in 10,000 of carbon dioxide be brought into equilibrium with water, the aqueous solution will contain three volumes of gaseous carbon dioxide in 10,000 volumes of water. That is, in 10 litres of water there will be dissolved 3 c.c. of carbon dioxide. Now, since 44 grm. of carbon dioxide gas measure at normal temperature and pressure 22,000 c.c., this means that 3 c.c. of carbon dioxide weigh approximately 6 mgrm.; 6 mgrm. of carbon dioxide in 10 litres, therefore, represents a concentration of 6 parts in 10,000,000 parts of water. It is the carbon in the carbon dioxide which is utilised in formation of the organic carbon compounds of the plant, and this stands to carbon dioxide in the relationship of 12 to 44, so that the concentration of assimilable carbon becomes reduced to about 1 part by weight in 6,000,000 parts of water.

So far as nitrogen assimilation is concerned, the amount of nitrogen required by plants is not on the average more than about 5 per cent. of the required carbon assimilation, so that, other factors, such as coefficients of distribution between air and water, being taken as equal, a concentration of nitrites or nitrates in the atmosphere or cell-sap about equivalent to 1 part of nitrogen in 120,000,000 parts of water ought to supply sufficient for assimilative purposes.

In a series of twenty experiments it was shown that both rain and dew invariably contain nitrites, but these slowly diminish, so that water drawn from a reservoir by a service tap gives a quite negative result when tested by the diazo-reaction for nitrites. If, however, another portion of this same sample of water be exposed, either in the open or within a transparent quartz container, to sunlight or an artificial source of light rich in short wavelengths,* in one or two hours a strong reaction is given for nitrites. In this manner, by testing water before and after exposure, the presence and relative amounts of nitrite and nitrate may be determined. Similar changes, only greatly diminished quantitatively, are obtained when the exposures are made in glass vessels, showing that it is the ultra-violet waves which are most potent. That this result is due to conversion of nitrates into nitrites, and not conversion of dissolved nitrogen and oxygen, is shown by the fact that it does not occur with distilled water holding air in solution; but if a small amount of potassium nitrate, say, one part by weight in 10,000 parts, be added, an intense reaction occurs on insolation. In several experiments it was shown that the presence of green leaves from different types of plants diminished the amount of nitrites present after exposure, as contrasted with control flasks alongside containing no green leaves.

---

* At the time the experiments on the action of rays from the quartz mercury arc light upon nitrates were made it was thought they were original, but since the paper was written two references have been discovered to experiments made by observers in France, who have approached the subject from quite a different standpoint. The first observer, M. Lombard, 'Comptes Rendus Acad. des Sciences,' vol. 150, p. 227 (1910), found that when tap-water or dilute solutions of nitrates were exposed to this source of light nitrites were formed. This was later confirmed by D. Berthelot and H. Gaudechon, 'Comptes Rendus Acad. des Sciences,' vol. 152, p. 522 (1911), but neither communication speaks of the importance in nature of this change, or shows it occurs with sunlight, or draws attention to the occurrence in green leaves, and the activation thereby produced. Attention is mainly directed to the cause of the sterilisation of water by exposure to ultra-violet light.

It is clear from these experiments that nitrates taken up by the rootlets of plants from the soil can be converted into the more reactive nitrites in the green leaf with absorption of solar energy; simultaneously, non-nitrogenous organic bodies are being built up in the same situation, which suggests that by interaction under the influence of light protein synthesis as well as carbohydrate may occur in the green leaf.

The presence of nitrites and nitrates in rain and dew indicates their occurrence in atmospheric air, and this was ultimately proven in a series of experiments which showed that the main portion of the oxidised nitrogen from air is found in water, after bubbling air through it, as nitrate and not as nitrite.

Great care is required in order to give a rigorous proof of this, because the condition of the absorbed substances from the air may be modified in the act of collection if light be not carefully excluded, and nitrate from the air be changed by insolation into nitrite. This fact first emerged from a series of experiments intended to study the relative amounts of nitrite in air by day and by night, when apparently the interesting result was obtained that there was practically no nitrite in night air, but a considerable amount in day air. Just then the effect of light in converting nitrates into nitrites was learnt, and a repetition of the experiment was made, using a blackened bottle with distilled water as absorbent. This distilled water had been twice distilled, and was so free from nitrate that it gave no Griess-Ilosvay reaction even after prolonged exposure to ultra-violet light. The result now obtained was that, whether the air were bubbled through by day or by night, only a very slight reaction for nitrites was obtained; but, on now exposing to sunlight this distilled water through which air had been bubbled in darkness, whether by day or by night, a strong reaction was obtained in each case, showing that oxidised nitrogen is present always in air both by day and by night.

It is not possible to conclude that the relative amounts of nitrate and nitrite in bubbled air give an indication of the relative amounts of the two oxides of nitrogen in the air; for if the absorption be attempted in the presence of light there will be a reduction to nitrite, and if in darkness, the great volume of oxygen simultaneously bubbled through may have oxidised nitrite to nitrate, so that there is a labile equilibrium between a given degree of light exposure and nitrates, nitrites, and oxygen.

Attempts are at present being made to follow up the earlier stages of nitrogen fixation from the atmosphere. The light of the sun in the upper strata of the atmosphere, where the ultra-violet has not yet been absorbed, must produce vast amounts of ozone, and these disappear as the earth's surface is approached; in so disappearing, the ozone may oxidise nitrogen and give nitrites and nitrates. Whatever their source, these nitrites are most reactive with organic substances and destructive to micro-organisms, and many of the natural bleaching, deodorising, and sterilising activities in air and water which have been hitherto ascribed to ozone and hydrogen peroxide are more probably due to nitrites.

Summary.

Dilute solutions of nitrates exposed either to sunlight or to a source of light rich in light-energy of short wave-length (such as light from mercury vapour arc enclosed in silica) undergo conversion of nitrate into nitrite.

There is an uptake of chemical energy in this reaction transformed from light-energy as in formation of organic carbon compounds in foliage leaves; it is to be added to the relatively small number of endothermic reactions known to be induced by light.

Interposition of a layer of glass between source of light and solution of nitrate greatly slows the reaction, showing that the most effective rays are those of short length.

When green leaves are immersed in nitrate solution, comparatively little nitrite accumulates, indicating that nitrites are rapidly absorbed by the green leaf. Nitrates taken up by plants from soil would, in presence of sunlight, be changed to nitrites, which are much more reactive than nitrates. This indicates that the early stages of synthesis of nitrogenous compounds are carried out in the green leaf and aided by sunlight.

Rain-water collected for considerable time contains no nitrites, all having been oxidised to nitrates, but if exposed to bright sunlight or ultra-violet light for a few hours a strong reaction for nitrites is always obtained.

Freshly collected rain-water or dew always contains a mixture of nitrites and nitrates, as shown by the nitrite test appearing without any previous treatment of the water and the great enhancement of this on exposure to strong illumination.

Air bubbled through nitrite- and nitrate-free distilled water gives a mixed reaction afterwards when the water is tested for nitrites and nitrates, showing presence of both forms of oxides of nitrogen in air.

There is no hydrogen peroxide or ozone in air at surface level. The fresh odour in open air, commonly referred to as "ozone," is probably nitrogen peroxide, which at high dilutions has the odour of ozone. The oxides of nitrogen are probably formed by the action of sunlight, rich in ultra-violet rays, in upper regions of the atmosphere upon air and aqueous vapour.

Attention is drawn to the importance of these actions of light in purification of air and water, and enrichment of soils and waters by this continuous supply of matter essential to organic growth, the energy of which, like that for upbuilding of non-nitrogenous organic compounds, comes from sunlight.

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(1) Moore and Webster, 'Roy. Soc. Proc.,' B, vol. 87, p. 163 (1913).

(2) Moore, *ibid.*, 1914, p. 556.

(3) Moore, 'Recent Advances in Physiology and Bio-chemistry,' edited by Leonard Hill, p. 29, *et seq.*, and p. 131. Arnold, London, 1906.

(4) Mendeléeff, 'Principles of Chemistry,' translated by Kemensky and Pope, vol. 1, p. 287. Longmans, London, 1905.

(5) Ilosvay, 'Bull. Soc. Chim.' [3], vol. 2, p. 666.

(6) Ilosvay, 'Ann. Chem. Pharm.,' vol. 124, p. 1 ; vol. 186, p. 2.

(7) Hayhurst and Pring, 'Chem. Soc. Trans.,' vol. 97, Pt. I, p. 868 (1910) ; Pring, 'Roy. Soc. Proc.,' A, vol. 90, p. 204 (1910), and Strutt, 'Roy. Soc. Proc.,' A, vol. 94, p. 260 (1918).

(8) Sutton's 'Volumetric Analysis,' 10th edition, p. 470. Churchill, London, 1911.

Action of Light Rays on Organic Compounds, and the Photo-synthesis of Organic from Inorganic Compounds in Presence of Inorganic Colloids.

By Benjamin Moore, D.Sc., F.R.S., and T. A. Webster.

(Received December 11, 1917.)

(From the Department of Applied Physiology and Hygiene, Medical Research Committee.)

In a former paper by the present authors* it was shown that sunlight in the presence of certain inorganic colloids, and notably of commonly occurring substances, such as colloidal ferric oxide or hydrate, possesses the power of acting upon water and carbonic acid, and yielding the energy necessary for the production of formaldehyde, from which carbohydrates and other organic constituents found in plants and animals might be built up.

In a later paper it was shown by Moore† that such inorganic compounds of iron occur associated in the chloroplasts of green plants, and especially marked in the lowlier green organisms, and hence that such photo-synthetic processes in which inorganic iron salts played the part of energy transformers might be regarded as taking a part in normal photo-synthesis in the plant.

The present paper records a continuation of these experiments. The source of light energy instead of sunlight was often the light of a mercury vapour lamp in a silica tube on account of the difficulty from weather conditions of using sunlight during the greater part of the year. Such a lamp gives certain lines of light in the ultra-violet range possessing a good deal of energy, and this part of the spectrum is the most powerful in producing the effects recorded, for it suffices to expose the solutions in glass tubes instead of silica tubes, or to interpose even a thin plate of mica between the source of light and the solution, in order to cut off nearly all of the effect.

As in the previous paper the test used for the presence of formaldehyde in the experiments with inorganic catalysts was that introduced by Schryver‡ in which a pink coloration is produced in presence of formaldehyde by phenyl-hydrazine and potassium ferricyanide in acid solution. The test is so delicate that it shows quite clearly one part in 2,000,000 of formaldehyde, and we have found it most satisfactory with continued use. Some attempt has been made in certain of our experiments to obtain roughly quantitative results by colorimetric comparison with diluted solutions of formaldehyde used as controls.

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* 'Roy. Soc. Proc.,' B, vol. 87, p. 163 (1913).

† 'Roy. Soc. Proc.,' B, vol. 87, p. 556 (1914).

‡ 'Roy. Soc. Proc.,' B, vol. 82, p. 226 (1910); Report No. 9, Inspector of Food's Department of the Local Government Board.

Three types of experiment have been carried out, viz.: (A) a considerable number of inorganic salts in dilute solution in water, and in either crystalloidal or colloidal solution, have been tested as to their activity as transformers in the action of light on water and carbonic acid; (B) the action of light upon formaldehyde at higher concentration has been investigated; and (C) the action of light upon organic bodies of more complex nature, such as carbohydrates, proteins, vegetable and animal juices and extracts, and other substances of bio-chemical interest has been studied. The results will be described in corresponding sections of this paper.

A. *Photo-synthesis by Inorganic Transformers.*

In the previous work it had been found that oxide of uranium in the colloidal state gave a far greater effect than the crystalloidal salts, and the inference was drawn that the state of aggregation of the colloid was favourable to the photo-synthetic reaction. Accordingly in our earlier experiments with iron compounds, colloidal ferric oxide was employed. Continued investigation of the iron compounds has, however, shown that the size of the solution aggregate may easily become too great, and that there is a certain degree of aggregation at which the catalytic action has an optimum value.

Thus, while dialysis of uranium oxide gives a condition in which the photo-synthetic effect is much greater than that obtained with an equal concentration of uranium nitrate in crystalloidal condition, dialysis of iron compounds on the other hand gives a condition in which less effect is obtained than with an equal concentration of ordinary ferric chloride.

It is to be remembered, however, that there is often in the case of ordinary solutions of salts of heavy metals a good deal of complex formation approaching a colloidal condition. This is shown, for example, in the case of solutions of cupric salts by the apparent lack of agreement between the molecular weights as deduced by the freezing-point method, and the degree of ionisation as given by conductivity determinations. It is shown in the case of ferric chloride solutions by the darkening in colour of dilute solutions on warming, approaching the colour shown by colloidal iron solutions, and persisting after the heated solution has cooled again, for prolonged periods of time. Also, if a dilute solution of ferric chloride be boiled a precipitation of a part of the iron as ferric oxide is obtained, and the precipitate remains permanent on cooling although the period of boiling is far too short to remove a corresponding amount of hydrochloric acid. All these facts demonstrate that there are ions and molecules in the ferric chloride solution of greater molecular complexity than the mono-molecular condition.

The explanation then of the greater effect with undialysed ferric chloride, as contrasted with colloidal ferric oxide solution, is that the mass of the complex molecule in the ordinary ferric chloride solution is that which can best take up the light vibrations and absorb the energy, whereas in the colloidal ferric oxide solution the solution-aggregate has become too heavy to take up the light-energy and convert it into chemical energy.

On the other hand, with the uranium salts, the optimum solution-aggregate to act as a transformer for light-energy lies nearer that found in the dialysed solutions than in the ordinary uranium nitrate solutions, and accordingly the former show a higher photo-synthetic activity.

Silicic acid and its salts show the same kind of effect as the uranium compounds, for dialysed silicic acid solution gives a strong photo-synthetic action, while both sodium silicate solution and undialysed silicic acid solution are inactive.

There appear, therefore, to be two factors in the production of photosynthetic activity by a given light source, viz., (1) the specific character of the inorganic catalyst or transformer, (2) the degree of its molecular aggregation in solution.

In view of criticisms that have been made as to the necessity of inorganic catalysts in the solution, and as to the possibility of the ultra-violet rays producing the synthetic effect when passed into pure water charged with carbon dioxide, as also the view that the formaldehyde obtained might be produced from the minute traces of organic matter in the dialysed solutions and not synthetically from carbon dioxide and water, the following critical series of experiments was carried out.

1. Water alone, freshly re-distilled, was saturated by a stream of carbon dioxide and then exposed in a quartz test-tube during the whole of a bright summer day on the roof to direct sunlight; alongside it in a similar quartz test-tube was exposed a 1-per-cent. solution of ferric chloride also saturated with carbon dioxide, and a third test-tube filled with 1-per-cent. ferric chloride saturated with carbon dioxide was kept in a dark cupboard.

Tested at the end, after distilling away from the iron salts, the distilled water tube and dark ferric chloride tube gave a negative result, while the tube of ferric chloride exposed to sunlight in presence of carbon dioxide gave a bright pink colour with the Schryver's reagent corresponding to about 1 in 500,000 of formaldehyde.

2. A solution of 1-per-cent. ferric chloride was made up with distilled water which had been freshly boiled and was free from carbon dioxide; this

was exposed during a whole week of bright sunshine in June, 1915, on the laboratory roof, being contained in a quartz test-tube. It was then distilled and tested with negative results.

These experiments clearly show that an inorganic energy-transformer is necessary, and that carbon dioxide alone in aqueous solution in sunlight does not form formaldehyde; secondly, that the formaldehyde is not a decomposition product of traces of more highly organised substances, but is actually built up by the inorganic colloid absorbing the energy of the sunlight and so becoming activated and reacting on the water and carbon dioxide, transferring the energy and producing formaldehyde.

Both the elements hitherto described, viz., uranium and iron, form higher and lower oxides, and it might, therefore, be urged that the higher oxide became reduced by the energy of the sunlight to a lower oxide with greater energy content, and that this lower oxide parting with its acquired energy to the water and carbon dioxide formed the formaldehyde to which the energy of the sunlight was thus indirectly transferred. Such a view is of interest because similar changes do actually occur in certain life processes, where various types of micro-organism, such as iron organisms, sulphur organisms, and nitrogen-assimilating or carbophilous organisms carry out similar energy transformations. The so-called iron bacteria are capable in darkness, as within an iron water-cistern or water-main, of utilising the energy of metallic iron or ferrous oxide, when given out in the process of oxidation to the ferric condition. The organism is enabled by a linked reaction to utilise for building up from carbon dioxide and water those reduced organic substances which form its body material. Similarly, the philothionic organisms are capable of utilising the energy of sulphur, or reduced sulphur compounds, to build up organic carbon compounds, and the nitrogen-assimilating organisms fed with organic carbon compounds can link up to the endothermic reactions necessary to convert the atmospheric nitrogen into ammonium salts or nitrites, and from these build up proteins.

In all cases where the energy of light is absent, however, there must evidently have been previously a light-transforming reaction at some earlier period of history, for without this the metal or lower oxides, or sulphur or sulphide would never have been formed upon which the organism not utilising light depends for its store of energy. In a world cooling down from red-heat, in presence of free oxygen in its atmosphere, all these substances would have been completely oxidised, and so the immense world deposits of pyrites and ferrous oxide and such-like reduced substances, just like coal, shale and petrol, must have originated from previous life processes, accompanied by energy transformations in presence of sunlight.

It is accordingly of some interest to enquire whether such transformations of sunlight into chemical energy by inorganic transformers are associated with a temporary chemical change from a higher to a lower oxide, or whether the change is a surface action in which the light-energy is converted into chemical energy at the surface of the colloidal aggregate.

Our experiments favour the latter view, for if the lower oxide reacted with the water and carbon dioxide to form formaldehyde, then a greater reactivity might be expected when ferrous salts were employed instead of ferric salts; this is, however, not the case, for we have found ferrous salts to be entirely inert, nor have we been able by titration with permanganate to show any formation of ferrous salts when ferric salts are exposed in aqueous solutions in presence of carbonic acid. Moreover, many of the active inorganic transformers which we have lately investigated, as recorded below, do not form higher and lower oxides under the conditions in which we have used them. It is therefore probable that the energy transformation is one induced by the energy of the light at the surface of the colloid particle upon which probably carbon dioxide concentrates.

A number of other solutions were then tested after exposure to the rays from a quartz mercury vapour arc. In addition to the ferric salts and uranic salts, strong positive results were obtained with 1-per-cent. solutions of dialysed silicic acid, and with a 1-per-cent. solution of beryllium chloride ; less active solutions, but distinctly positive in 1 per cent. solution, were copper chloride and sulphate, nickel chloride, palladium chloride, manganese chloride, erbium chloride. Negative results were given by ferrous sulphate and chloride, sodium silicate, and undialysed hydrochloric acid solution of silicic acid, zinc chloride, cobalt chloride, potassium chloride and chromate, barium chloride, aluminium chloride, borax and telluric acid.

The above-named solutions were exposed in each case for a period of four to five hours in transparent quartz test-tubes, at a distance of 7 cm. from a mercury vapour arc in a quartz tube.

B. *Action of Sunlight and of Ultra-violet Light upon more Concentrated Solutions of Formaldehyde.*

When formaldehyde is once formed by the action of sunlight from carbon dioxide and water, practically all the energy necessary for formation of carbohydrates by the condensation of formaldehyde groups has been absorbed; for although the heat of combustion of formaldehyde has never been determined, judging from analogy in similar cases, the heat of combustion of six gramme-molecules of formaldehyde should be almost equivalent to that of one gramme-molecule of a hexose sugar. For example, the energy of formation, or combustion, of two gramme-molecules of any hexose is almost equivalent to that of one gramme-molecule of a biose or disaccharide, and all sugars and starches possess almost equal stores of chemical energy in equal masses.

As has been pointed out by Moore,* it is under such conditions that the typical reactions in living systems occur. When the total chemical energy of the reacting substances on either side of the equation is large, but the difference on the two sides is small, then typical reversible reactions are seen with the equilibrium point a considerable distance removed from either end-point.

This condition of affairs so characteristic of bio-chemical reactions is due to a labile balance between osmotic energy and chemical energy, and it is for this reason that conjugation with formation of more complex molecules is favoured by higher concentration, whereas dilute solution favours cleavage into simpler molecules. This point is of great importance in biological synthesis, and for understanding reversal of action within and without the cell, and as it is not yet sufficiently appreciated, an example illustrating it may be adduced.

Taking the heats of combustion as determined by Thomsen, the following equation gives the heat of reaction in the change between glucose and maltose (K = 100 gramme-calories):—

$$\begin{array}{ccc} C_6H_{12}O_6 + C_6H_{12}O_6 & \rightleftarrows & C_{12}H_{22}O_{11} + H_2O - 33K, \\ (\text{Glucose}) + (\text{Glucose}) & \rightleftarrows & (\text{Maltose}), \\ 6737K + 6737K & \rightleftarrows & 13{,}507K \quad -33K. \end{array}$$

Thus the reaction is apparently a slightly endothermic one, as it runs from glucose into maltose (a rule which holds for all the similar reactions), but observe that as the reaction runs in this direction the osmotic energy of the system diminishes, for every two molecules of glucose passing out of the system are replaced by only one of maltose. Hence for every gramme-molecule of glucose passing into maltose a definite amount of osmotic energy is set free, which can be converted into chemical energy, and hence make an apparently endothermic reaction run without added energy from without. The amount of osmotic energy set free by the disappearance of the molecules of a constituent varies with the pressure at which it disappears, and therefore increases with the concentration of the solution,† and hence it came about that the first successful experimental proof of reversibility was given by Croft Hill* by the use of exceedingly concentrated sugar solutions, and that all such conjugations occur in concentrated solutions. Local concentration will have a like effect, and in living cells concentrations on surfaces and interfaces will produce such a result.

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* 'Recent Advances in Physiology and Bio-chemistry,' edited by Leonard Hill, pp. 19-40. Arnold, London, 1906.

† The thermodynamic proof of this is given by Moore in the articles quoted previously.

In many of the reactions of inorganic chemistry the differences in totals of chemical energy on the two sides possess a high magnitude at ordinary temperatures, as, for example, in the reaction between hydrogen, oxygen and water. Here the changes in osmotic energy are too insignificant to produce an appreciable effect, and so the reaction runs practically completely to one end or phase.

But, in the type of reaction with which we are here dealing of conjugation or cleavage where the chemical energy change is relatively small, the osmotic change becomes a powerful factor.

In the green cell of the living plant the formaldehyde can be condensed on an interface and there conjugate, although general concentration in the cell is kept at a low level. If it be sought to imitate this in a solution of formaldehyde, the concentration must be increased so that the decrease of osmotic pressure may yield energy to supply that required in conjugation, or assist energy supply from without, such as light energy, to increase the potential towards chemical union.

This is what has been done in the experiments here recorded, in which a reducing substance has been obtained by subjecting comparatively concentrated formaldehyde solutions to the light of the quartz mercury vapour lamp.

When six molecules of formaldehyde condense to form one molecule of a hexose, there is only one molecule of dissolved material contributing to keep up the osmotic pressure where there were formerly six, and a corresponding amount of osmotic energy has disappeared as such, and been utilised to yield the slightly higher content of chemical energy which the hexose possesses over that of the formaldehyde which went to form it. The energy so yielded will evidently be proportional to the osmotic pressure at which the formaldehyde molecules disappear, that is to say, to the concentration of the formaldehyde solution. The osmotic pressure represents the intensity or potential factor of the osmotic energy, and this comes into equilibrium with the intensity factor of the chemical energy, tending to disrupt hexose into formaldehyde and set energy free.

This osmotic energy supply is sufficient to yield the amount required, and hence, in this type of reaction, the light plays mainly the part of a catalyst, and not a provider of energy as in the synthesis of formaldehyde from carbon dioxide and water, or in the synthesis of nitrites from nitrates.

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* 'Journ. Chem. Soc.,' vol. 73, p. 634 (1898); vol. 83, p. 578 (1903).

Hence it arises that very dilute solutions of formaldehyde do not yield hexoses under the action of light, and that dilute solutions of sugars do not condense to form disaccharides. Conversely, if dilute solutions of the higher condensations, such as disaccharides or polysaccharides, be exposed to light, they split up into simple sugars, and these invariably yield formaldehyde, as will be shown in Section C.

The first synthetic sugars were obtained by Butlerow,[*] by Loew, and later by Fischer and Passmore by acting upon concentrated solutions of formaldehyde or other organic substances with caustic alkalies. Thus Loew used formaldehyde and milk of lime, and later freshly precipitated hydroxide of lead, Butlerow dioxymethelen and caustic potash, Fischer and Passmore acrolein bromide and baryta water.

Such powerful reagents accomplish the condensations and give origin to reducing sugars even in darkness and without need of external supply of energy. In the absence of the alkali which acts as a catalyst, the reaction towards equilibrium is held in check, or proceeds only at an infinitely slow rate, so that a concentrated solution of formaldehyde can be kept in the dark for an indefinite period without developing any reducing substances.

In nature, no such strong hydroxyl-ion concentration is found in plant tissues as that of the reagents used for synthesis of sugars *in vitro* as mentioned above; it is therefore interesting to obtain condensation with light exposure in absence of any high alkalinity.

At the time of our experiments we were unaware of any existing observations on the subject, but we have since discovered a paper by R. Pribram and A. Franke,[†] in which condensation of formaldehyde and formation of reducing substances was brought about by exposure of concentrated aqueous solutions to ultra-violet light, also a paper by an Italian observer, G. Inghilleri,[‡] who exposed a mixture of concentrated aqueous formaldehyde and 6 per cent. of oxalic acid in sealed glass tubes in sunlight for several months, and obtained a hexose which he identified as the sugar sorbose (inactive). In both these cases, although the authors do not comment upon it, the law, stated above, of condensation in strong solutions holds.

Formaldehyde itself reduces Fehling's solution in slight degree after long boiling, though not so readily as any hexose solution. It was therefore of importance to find, if possible, some indicator which would not be reduced by formaldehyde, but would be reduced by more strongly reducing organic substances. This was found in Benedict's solution, an alkaline solution of copper sulphate in presence of sodium citrate and sodium carbonate; this is not reduced at all even on prolonged boiling with formaldehyde, but a solution of formaldehyde after exposure to ultra-violet light reduces it readily, and the same effect is brought about, though much more slowly, by sunlight.

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[*] Butlerow, 'Liebig's Ann.,' Bd. cxx, p. 295; O. Loew, 'Berichte d. D. Chem. Gesellsch.,' Bd. xix, p. 141 (1886); Bd. xx, pp. 141 and 3039 (1887); Bd. xxi, p. 278 (1888); Fischer and Passmore, 'Berichte d. D. Chem. Gesellsch.,' Bd. xxii, p. 359 (1889).

[†] 'Berichte d. D. Chem. Gesellsch.,' Bd. xliv, p. 1035 (1911), and 'Monatsh. f. Chem.,' Bd. xxxiii, p. 415 (1912).

[‡] 'Zeitsch. f. Physiol. Chem.,' Bd. lxxi, p. 105 (1911), and Bd. lxxiii, p. 44 (1911).

This condensation reaction with formaldehyde differs from the synthesis of formaldehyde from carbonic acid and water, in that it does not require the presence of an inorganic activator. In the earlier experiments this was not known, and in these the reduction of the copper salt occurred around the colloidal silicic acid, added as an intended activator, showing the interesting fact that the sugar, or reducing substance, had been absorbed by the silica. Later experiments demonstrated, however, that the reaction proceeds with equal or greater rapidity when a solution of 4–5 per cent. of formaldehyde in water only is exposed in quartz tubes to ultra-violet illumination.

Experiment 1.—A solution containing 5 per cent. of formaldehyde and 0·97 per cent. colloidal silicic acid was exposed at 9 cm. distance to the rays from a quartz mercury vapour lamp for a period of 6 hours. At the end of the period a distinct reduction, chiefly in the precipitated silica, was obtained with Benedict's solution. A control, exactly similar, but kept in the dark, gave no reduction. A portion of control solution kept warm in darkness also gave no reduction.

Experiment 2.—Two quartz tubes were exposed alongside each other for a period of eight hours at 9 cm. distance from lamp; one contained 5 per cent. formaldehyde in water only, the other 5 per cent. formaldehyde in colloidal silicic acid solution. At the end, the tube with silicic acid reduced Benedict's solution, but to a much less degree than that containing formaldehyde alone, showing that dialysed silicic acid probably acts as an anticatalyst.

Experiment 3.—A sufficient concentration of the formaldehyde appears to be reached at about 5 per cent., in order to develop the maximum rapidity of formation of the reducing substance. Thus two quartz test-tubes, one containing 40 per cent. and the other 8 per cent. of formaldehyde, were exposed to the quartz mercury-vapour lamp, at the same distance (9 cm.) for the same period (three hours) in each case. Both gave quite a marked reduction, at least as great in the less concentrated solution as in the highly concentrated one. This result, and that of the succeeding experiments

(Experiment 4), does not invalidate the reasoning given above, for 2-per-cent. solution of formaldehyde is already a concentrated solution possessing as high a molecular concentration as a 12-per-cent. solution of a hexose, and the optimum has already been reached.

Experiment 4.—In a similar experiment, four quartz flasks of 250 c.c. each were filled respectively with 0·5, 1, 2, and 4-per-cent. solutions of formaldehyde, and exposed to ultra-violet light at 7½ cm. from the lamp. As a result of the greater volume in the flasks per unit of surface, the appearance of reducing substance is slower than in the quartz test-tubes, but the 2-per-cent. solution showed distinct reduction after seven hours' exposure; the stronger 4-per-cent. solution and the two more dilute solutions at this time gave no reduction. Tested again after 10 hours' total exposure, the ½-per-cent. solution was still negative, while the 1-per-cent. showed a faint reduction, the 4-per-cent. somewhat more, and the 2-per-cent. still gave the best reduction. The ½-per-cent. solution only begins to reduce after about 30 hours' exposure, when all the others are giving a copious reduction. Control flasks kept at 40° C. in thermostat showed no development of reducing power during this period.

Experiment 5.—The formaldehyde used in the above experiments had not recently been distilled, and contained para-formaldehyde. In order to test if the simple aldehyde, or its polymer, or both, gave the reducing substance under the action of the ultra-violet light, a portion of the commercial 40-per-cent. solution was distilled, and the first portion coming over, as well as the solid polymer left behind in the retort after distilling over the greater part, were separately made up in aqueous solution, and exposed to light in about 4-per-cent. solution. The solid was dissolved in distilled water, and the distilled aldehyde was diluted with distilled water. Both were tested with Benedict's solution before exposure, and neither gave any reduction. The two solutions in two quartz test-tubes were then exposed for four hours at 8 cm. distance, and, on testing, a copious reduction was obtained in both cases.

Experiment 6.—This experiment was mainly designed to locate the wave-length of the light causing the condensation. Two test-tubes were taken, one made of transparent quartz, the other of ordinary soft glass; the dimensions and thicknesses of wall of the tubes were about the same, the capacity of each being about 35 c.c. Each test-tube received 25 c.c. of the same 4-per-cent. aqueous solution of formaldehyde which had recently been distilled, and the two tubes were placed in equally favourable positions alongside each other at about 5 cm. distance from the quartz tube of a mercury-vapour lamp. At the outset the formaldehyde gave no reduction of

Benedict's solution. After three hours' exposure the contents of the quartz tube gave a fair reduction of Benedict's solution, while those of the glass tube still gave a complete negative with this test solution. At the expiry of 6½ hours' exposure the contents of the quartz tube gave a copious reduction, but the contents of the glass tube had not yet developed any reducing substance. At the end of 12 hours the contents of both tubes showed reduction, those of the glass tube to about the same extent as had been obtained in the quartz tube in three hours, while the contents of the quartz tube now gave a heavy precipitate of cuprous oxide when tested by the Benedict's solution.

The remainder of the contents of the quartz tube were distilled at ordinary atmospheric pressure almost to dryness, leaving a white solid residue in the distilling flask. The residue was taken up with warm water, in which it readily dissolved, and made up to the original volume. Both distillate and residue were then tested with Benedict's solution, and both were found to reduce it readily, the residue containing apparently somewhat more reducing substance than the distillate. Again, both distillate and residue reduce neutral silver nitrate solution and mercuric chloride solution. This experiment shows that the short wave-lengths are the most powerful, and that the limit lies at about the shortest wave-lengths transmissible by glass.

Experiment 7.—In a somewhat similar experiment two quartz test-tubes were taken, of the same dimensions as above, and the same concentration of formaldehyde was employed, but around one of the tubes as thin a sheet of mica as could be split off was folded so as completely to surround the quartz, and held in position by two loops of thread at top and bottom. The mica was so thin that it readily folded over the test-tube (about 3 cm. in diameter) without cracking. Yet this thin layer of mica was so effective a screen that there was not a trace of reduction after a 12 hours' exposure similar to that given above in glass; the contents of the unshielded quartz tube showed a distinct reduction after two hours' exposure.

Experiment 8.—Only preliminary experiments have hitherto been made towards the identification of the substance or substances giving the reductions with these metallic salts. It is natural to suppose that there should be representatives of intermediate condensation products lying on the path between formaldehyde and the hexoses. Pribram and Franke, in the papers above referred to, believed they were able to identify glycolaldehyde arising from the condensation of two molecules of formaldehyde, and Inghilleri claims to have isolated the racemic sorbose by means of its osazone.

It was evident to us that we were dealing with a complex mixture, and, although there is a quite distinct effect with phenyl-hydrazine in acetic acid solution, quite different from that of formaldehyde, and giving abundance of coloured precipitate, we have not yet succeeded in isolating a crystalline osazone, though we have on several occasions obtained crystals mixed with amorphous smears.

In order to prepare large quantities of material, four quartz flasks, each of about 300 c.c. capacity, were completely filled with a 4-per-cent. solution of formaldehyde, and exposed at a distance of about 4 inches from the lamp for several hours daily for a week. The result was disappointing, on account of the slowness of action. The effect appears, like many effects of light, even in clear solutions, to be all concentrated within a comparatively thin layer lying next the incident surface. Accordingly, as in these larger vessels the volume increases much more rapidly than the exposed surface, the concentration of reducing substance in the solution progresses at a correspondingly slow rate. At the end of the week there was a fair amount of reduction in all four flasks, but not more than would be obtained in a small quartz test-tube with a single day's exposure. In continuation, four quartz test-tubes, each of about 30 c.c. capacity, were filled with part of the contents of one of the flasks, and exposed at 5 cm. distance for two days. The contents were mixed and used for the following experiments:—

Several attempts to obtain an osazone were made by heating with excess of phenyl-hydrazine and acetic acid. The unchanged formaldehyde interfered, and although attempts were made to overcome this by fractional precipitation, the most that could be obtained was occasionally small microscopic tufts of crystals, mixed with oily and amorphous material.

Changes due to the exposure are evidenced by the deep orange colour and orange precipitate obtained on boiling with the phenyl-hydrazine in the case of the exposed solution, while similar treatment of the unexposed formaldehyde gave only a pale yellow colour in both solution and precipitate.

An attempt was made to remove the unchanged formaldehyde by forming the addition compound with aniline, but unfortunately the reducing substance also precipitated with the aniline. When added to exposed formaldehyde solution aniline causes a white precipitate as it does with unexposed formaldehyde, but the latter precipitate remains white on heating, while that given by the exposed solution turns a dark orange colour when heated. The filtrate from the aniline precipitate no longer reduces Benedict's solution, even when concentrated on the water-bath, neither does the aniline precipitate reduce. It was not possible to separate from the precipitate anything but formaldehyde and aniline.

Exposed formaldehyde solutions turn yellow when concentrated by distillation either at atmospheric or reduced pressure and leave behind a yellow syrup with a bitter taste which strongly reduces Benedict's solution; unexposed formaldehyde solutions leave a smaller residue of white paraldehyde.

Experiment 9.—The condensing action of light is aided by slight alkalinity. Three quartz test-tubes were taken: in the first was placed a mixture of equal volumes of 4-per-cent. formaldehyde solution and water, in the second and third a mixture of equal volumes of 4-per-cent. formaldehyde solution and of a 1-per-cent. solution of sodium carbonate (Na_2CO_3). The first and second were exposed for three hours at a distance of 3 cm., while the third was kept in darkness in an incubator at approximately the same temperature as that caused by the irradiation, viz., about 50° C. At the end of the period the unexposed solution gave no reduction with Benedict's solution, and the tube contents exposed in presence of the alkaline carbonate gave practically double the intensity of reduction of those equally diluted with water. Tested again at the end of 15 hours the contents of the tube kept warm in darkness still gave no reduction, while the other two now gave each a heavy reduction. Even after an additional period of three days in the incubator the dilute formaldehyde and sodium carbonate solutions kept in darkness gave no reduction with Benedict's solution.

C. *The General Formation of Formaldehyde by the Action of Light upon Organic Substances of Bio-chemical Origin.*

The series of experiments leading to the generalisation made in this section were induced by an attempt to construct an emulsion of chlorophyll in extractives from green leaves along with colloidal ferric hydrate so as to produce an artificial system resembling that present in the chloroplast of the green plant, which might then be exposed to light and tested for production of formaldehyde. For this purpose a quantity of grass leaves was washed with water and then extracted with absolute alcohol. The filtrate was allowed to remain at room temperature in a desiccator over sulphuric acid until the alcohol had evaporated and a green extract remained behind. This was rubbed up and shaken into an emulsion with a colloidal solution of ferric oxide. This emulsion was saturated with carbon dioxide and exposed for about four hours to the light of the quartz mercury vapour lamp. At the end the chlorophyll had bleached, and the ferric hydrate had coagulated so that the whole could be filtered. The result was surprising as to the intensity of the formaldehyde return; when the Schryver test was applied there was at once a deep pink colour produced. Even after a twenty-five-fold dilution a reaction was obtained of about 2 parts of formaldehyde per 1,000,000, showing an amount of about 1 part in 20,000 in the original emulsion. This result was not, however, due to the combined action of chlorophyll and colloidal ferric hydrate, for a similar intense result was obtained when an exposure was made of an emulsion of chlorophyll extract alone in distilled water and without any carbon dioxide.

It follows that the formaldehyde must have originated either from the chlorophyll or the other organic substances in the green leaves taken out by the alcohol along with the chlorophyll.

Additional experiments showed us that practically any complex organic substance of biological origin yields formaldehyde when exposed to the action of ultra-violet light, and also—though more slowly—when exposed to sunlight. Solutions or emulsions were exposed of all the commoner sugars (such as glucose, fructose, maltose, lactose, saccharose), of glycogen, starch, glycerine, egg-albumen, milk, and various vegetable juices. After three to four hours' exposure and subsequent filtration or distillation those all showed the presence of formaldehyde in varying amount. The more transparent solutions gave the more intense reactions, and amongst these the sugars were most rapidly disrupted by the action of the light. The action appears to be one of successive hydrolysis; thus, for example, cane sugar is first inverted, and after an hour or two gives a strong reduction with Benedict's solution. The reaction takes place also with sterilised sugar solutions, in sealed glass tubes, exposed to bright sunlight, but the speed of reaction is much less than when a quartz container is used, and the exposure must be continued for several days.

The action of sunlight, and of ultra-violet light, on organic substances has been studied by many observers* and on a vast number of substances. The production of formaldehyde in a certain number of instances has been noted by different experimenters, but no one appears hitherto to have noticed the generality of the appearance of formaldehyde when any complex substance of vegetable or animal origin is exposed to light vibrations of short wavelength. This may be due to the employment by us of a more delicate reagent, and to a systematic testing for the formaldehyde in all cases.

Certain of the observers, such as C. Neumann, and D. Berthelot and Gaudechon, appear to consider a catalyst as essential to the reaction, and for this purpose have used salts of uranium. The reactions of de-duplication of these organic substances in dilute solutions take place, however, quite readily without a chemical catalyst. The reactions are exothermic, and the light itself acts as the catalyst.

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* The papers on the subject are too numerous to quote separately; a key to the literature will be found in the following:—Ciamician and Silber, 'Berichte d. Deut. Chem. Gesellsch.,' from 1901 onward; 'Atti Real. Accad. Lincei,' from 1901; C. Neumann and Co-workers, 'Bio-Chem. Zeitsch.,' from 1908 onward; V. Henri and Co-workers, 'Comptes Rendus Acad. des Sci.,' and 'Comptes Rendus Soc. de Biol.,' from 1910 onwards; and D. Berthelot and Gaudechon, 'Comptes Rendus Acad. des Sci.,' from 1910 onwards.

This production of formaldehyde has several important and interesting relationships which may now be pointed out.

In the first place, it has a practical bearing on all enquiries as to the presence of formaldehyde in green leaves exposed to light, or of chlorophyll solutions, or artificial schemata of various types, exposed to light and afterwards tested for formaldehyde to elucidate the functions of the chloroplast in the green leaf. Many observers throughout the past generation have laboured at proving the presence of formaldehyde in green leaves exposed to light, but if it is so that practically any organic substance of bio-chemical origin exposed to light develops formaldehyde, then the presence of formaldehyde in green leaves furnishes no proof of its synthesis by sunlight from carbon dioxide and water. The same holds for all the schemata, for these always contain substances from which formaldehyde could arise. But even although no other organic substance save chlorophyll, or any mixture of pure chlorophylls, were present and yet formaldehyde were formed in abundance on exposure to light, this would be of no avail as a proof of photo-synthesis of formaldehyde from the inorganic, for the same change would happen in a solution of cane sugar, and there is no proof that the chlorophyll is not simply behaving like a legion of other organic compounds and yielding formaldehyde by its own decomposition.

It has recently been shown by Jörgensen and Kidd* that pure chlorophylls exposed as a suspensoid sol in water to light in presence of oxygen, at first bleach, and then yield formaldehyde. When exposed in presence of nitrogen only or a full atmosphere of carbon dioxide no formaldehyde was produced. Under natural conditions in the leaf and under all conditions of exposure to light of artificial schemata, used by previous observers, there has always been present atmospheric oxygen, so that this appearance of formaldehyde from pure chlorophyll emulsions after exposure to light confirms the view expressed here as the result of our experiments.

In the second place, the general production of formaldehyde when these substances resolve themselves under the influence of light into simpler forms possesses a teleological bearing, for if in the uptake of solar energy the first storage from the inorganic be in the stage of formaldehyde, it would be very probable that in the process of unbuilding this step should be retraced.

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* 'Roy. Soc. Proc.,' B, vol. 89 p. 342 (1916).

The general production of formaldehyde by the action of light on biochemical materials may also stand related to the important lethal effects of sunlight and ultra-violet light upon micro-organisms, which is seen in the sterilising action of sunlight upon many pathogenic organisms and in the similar use of ultra-violet light installations for sterilisation purposes.

The relationship of the lethal effects to the wave-length of the light has been studied by many observers. Downes and Blunt* showed, at an early period, not only that both direct and diffuse sunlight inhibited the appearance and slowed the growth of organisms self-sown in Pasteur's cultivation fluid, but that this action did not appear when the cultures were preserved behind red or yellow glass screens, while blue or violet glasses allowed light to pass which possessed as much deterrent action as light through clear glass. Marshall Ward† was the first to invent an ingenious method of making the organisms record their own destruction, which has again been independently re-discovered by two other sets of investigators at intervals of several years. The method consisted in throwing a spectrum, obtained by sending sunlight, or the light of electric arcs in air, through a quartz spectroscope, upon an agar-agar plate sowed over with the organism. A number of slots were cut out on the covering lid of a shallow plate like a Petri dish, and some of these were covered with quartz and others with thin glass strips. The spectra were directed through these on to the agar-agar culture. The remainder of the surface was protected by an opaque cover of tinfoil. After exposure for 12 hours the plate was incubated for four days and then the results were photographed. When the glass was interposed the only area of destruction was that of the blue and violet, but when the quartz only intervened between source of light and organisms the destruction passed far on into the ultra-violet region. A quite similar method was used by Barnard and Morgan,‡ who found the lethal action in the ultra-violet so intense that the bright spectral lines in the ultra-violet were mapped out as clearly almost as on a photographic plate. These authors also determined the wave-lengths of the lethal zone of the spectrum, and found its limits to lie between 3287 and 2265Å. Quite recently the work has been independently repeated with similar results by Browning and Russ.§

Some of the authors quoted, especially Marshall Ward, consider the nature of the chemical reaction involved. Marshall Ward draws attention to the oxidising action of blue light upon oils, and considers it probable that the effect is due to such an oxidation of the fat-reserve and not a direct action on cell-protoplasm.

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* 'Roy. Soc. Proc.,' vol. 26, p. 488 (1877); vol. 28, p. 199 (1879).

† 'Roy. Soc. Proc.,' vol. 54, p. 472 (1893); other papers by Marshall Ward on this subject, 'Roy. Soc. Proc.,' vol. 52, p. 393 (1892); vol. 53, pp. 26 and 164 (1893); vol. 56, p. 345 (1894).

‡ 'Roy. Soc. Proc.,' vol. 72, p. 126 (1903); 'Brit. Med. Journ.,' November 14, 1903.

§ 'Roy. Soc. Proc.,' B, vol. 90, p. 33 (1917).

It is highly interesting that the chemical reactions upon substances of biochemical origin, above described, are also produced by the same short wave-lengths as those which occasion death of organisms, as is shown by the enormous decrease in activity when the light is screened by passing through glass or mica.

Now the substances present in the bodies of the organisms are of those organic types which yield formaldehyde, as shown above, when exposed to the action of light vibrations of the shorter wave-lengths. It is well known that formaldehyde in high dilution is poisonous to such living organisms and when produced nascently by the action of sunlight, or ultra-violet light, and probably at selective concentrations on interfaces, it is quite probable that the death of the whole organism might so be induced. The action of the light would be progressive upon the living cell just as it is within quartz containers upon the more complex organic substances, and would manifest itself in a continuous hydrolysis of the more conjugated to less conjugated substances. The first effect would be upon the state of colloidal aggregation of the system, but concurrently formaldehyde and other organic compounds of simple type would be set free.

It is noteworthy that formaldehyde and other simple related substances, such as would be the first stages in the evolution of the organic from the inorganic, are all highly poisonous to the much later product in evolution, namely, the living organism. Such simple substances are formaldehyde, formic acid, oxalic acid, hydrocyanic acid, methylic and ethylic alcohols, hydrogen peroxide, and the simpler nitrites and nitriles; all these are poisonous to the highly organised and labile colloids of the bioplasm, and, probably on account of that very property which makes them essential in the first stages of organic evolution, namely, their high reactivity and the ease with which they take part in additive reactions of organic substances. For this reason they must undergo change in any living cell whilst still at high dilution, or else they so interlock into the labile system of organic colloids within the cell as to clog all metabolic change. Hence it is that the energy of light, which is essential to healthy growth and the upbuilding of organic material from inorganic, supplied in a wrong fashion and not shielded in its onset, may reverse these delicate processes and cause death and degeneration of living substance, instead of being the potent agency towards building up fresh material.

The subject is one of enormous and far-reaching importance. Blue light, as shown by Marshall Ward, is the most universal and potent natural purifier of our oceans and streams and our supplies of drinking-water. Light is also the agency which in spring, when the sun attains a certain altitude and less of the actinic light is absorbed by the atmosphere, penetrates the water of the ocean and lakes in sufficient intensity to stimulate the great outburst of vegetable plankton, which initiates the long sequence of swarms of animal life up to the fishes, and supports all the life of the seas. This is evident, because the spring outburst of floating plant life occurs before there has been any rise in the temperature of the sea-water. The bronzing of the skin, caused by exposure to bright sunshine, and the pigmentation of human races in tropical climates, is almost certainly a protective screen against these injurious rays, and Marshall Ward has pointed out that the pigments of those micro-organisms and fungi which can flourish in light always absorb these injurious rays, and allow passage to the reds, greens, yellows, and oranges, which are not injurious Even blue and violet pigments occurring in nature, when carefully examined spectroscopically, are found in many cases to absorb the violet and shorter-waved blues.

The same is true of the colours of flowers, and even of the green colouring matters of the foliage leaves, and it may well be that the function of the chlorophyll, which usually occurs as a thin layer like a skin over the chloroplast, is to temper and screen the light for the really effective transformer lying underneath.

The absence or great diminution of the blue and ultra-violet rays in hazy or sunless weather may also be of great importance in allowing the disease organisms of the higher plants to flourish unchecked, and it is such weather in autumn which often heralds the outbreak of disease and blights.

Summary.

The results are recorded under three sections:—(*a*) photo-synthesis by inorganic transformers; (*b*) action of sunlight and of ultra-violet light upon concentrated solutions of formaldehyde; (*c*) the general formation of formaldehyde by the action of light upon organic substances of bio-chemical origin.

In the first section, the reactions of a number of inorganic systems in presence of carbon dioxide and exposure to light are investigated, and it is shown that certain of these can build up formaldehyde while others are inert. The activity is shown to be related to the development of an optimum degree of colloidality, and is not due to formation of higher or lower oxides, but more probably to surface condensation on interfaces.

The second section deals with the condensation of formaldehyde to form reducing substances leading to carbohydrates, and discusses the conditions favourable for such condensations. The energetics of such a system are treated of in this section, and the effects of general or local concentration are considered. The equilibrium point in reversible reactions is shown to be dependent on concentration.

In the concluding section a general reversible reaction is described as a result of which formaldehyde rises in all intense reactions of light upon substances of bio-chemical origin. This reaction in presence of excess of light is an interesting reversal of the process by which all organic matter has been built up from inorganic sources.

The bearing of this process upon the germicidal action of sunlight, and the destruction of living organisms by ultra-violet light, is discussed, and it is pointed out that the simple organic products so formed are incompatible with the life-processes of living organisms, and so lead to their destruction.

Taking such a reaction as travelling in the reverse direction, it is shown that the building up of organic matter from inorganic must have been a necessary precedent to any existence of living organisms on the earth, and that all accumulations of reduced substances possessing stores of chemical energy must have arisen in this manner from storage of the energy of sunlight.

Growth of Trees, with a Note on Interference Bands formed by Rays at Small Angles.

By A. Mallock, F.R.S.

(Received December 1, 1917.)

But little is known about the growth of wood, little that is as to the times and rates at which the growth takes place.

When a tree is cut down, its age and growth in a season can be determined by the number and dimension of the annual rings, at any rate where the annual rings exist and are well marked; though it not infrequently happens that the rings are alternately weak and strong, so that some doubt may arise as to whether there have not been two periods of growth in one year.

Many tropical trees do not show annual rings at all, and in their case the age of the tree and its growth in a year cannot be found from an examination of a section.

It would, I believe, be an assistance in Forestry could some fairly simple means be found for measuring rapidly, *i.e.*, in a few days, or even weeks, the rate of growth of timber trees; and during the last summer I have made a few trials of such measurements, using an adaptation of an apparatus previously designed for observing the extension of cracks in buildings.

In both trees and building cracks the rate of extension is very small, though much greater in the former than the latter.

The increase in diameter of ordinary timber trees, as shown by the distance between the annual rings, varies largely with the species and the surrounding conditions, ranging from less than 0·1 up to 0·8 inch per year, or say from $\frac{1}{4}$ to $2\frac{1}{2}$ (or more) inches increase of girth. If this growth were continuous and uniformly spread over the year, the increase per hour would be between 0·000028 and 0·00028 inch.

Cracks in old buildings, on the other hand, may spread perhaps only at the rate of an inch in 1000 years or 0·0000001 (or one ten-millionth of an inch) per hour. With a good microscope there is no great difficulty in measuring lengths of 0·00005 inch, so that, as far as magnitudes are concerned, the hourly growth of trees could be quite well determined in this way; but to apply the necessary magnifying power in the position required would in most cases be inconvenient.

If, in place of ordinary optical magnification, interference methods are employed, so that the change in the girth of the tree is measured in terms of wave-lengths, much simpler apparatus will suffice; for in this case the change of the position of interference bands which are visible without, or with very little, magnification takes the place of micrometer measures made with high-power objectives.

There are many ways of producing suitable interference bands, but I will only mention the two which I have actually used. If two flat glass plates, A and B, one of which (say B) has a straight edge, are superposed, so that the straight edge of B rests on the surface of A, and if the surfaces of A and B are slightly inclined to one another, and are viewed by reflected monochromatic light incident normally or nearly so, the field will appear covered with parallel and equidistant light and dark bands, parallel to the edge of B, separated by intervals which are directly proportional to the wave-length of the light, and inversely as the angle between the plates.

If the angle is altered so that the $(n+1)$th band (say) occupies the place formerly held by the nth band, the distance between the plates at that place is altered by half a wave-length (in the case of soda light about the hundred-thousandth of an inch). With suitable means the shift of one-tenth of a band can be recognised, corresponding to an alteration of distance between the plates of a millionth of an inch. This is the plan suitable for detecting the extension of cracks.

For the much more rapid growth of trees, however, it is convenient to use an arrangement which demands a greater variation of angle to cause the same amount of shift in the bands, and this can be secured by merely forming the bands by light having a grazing instead of a normal incidence. For this purpose the plate A is replaced by a right-angled prism. The bands now formed are not equidistant and have several peculiarities which need not be here particularised. The theory is given in the note at the end of this paper. What is of importance for the present purpose is, that the alteration of angle between A and B necessary to shift one band to the position formerly occupied by its neighbour is more than ten times as great as when the incidence of the light is normal.

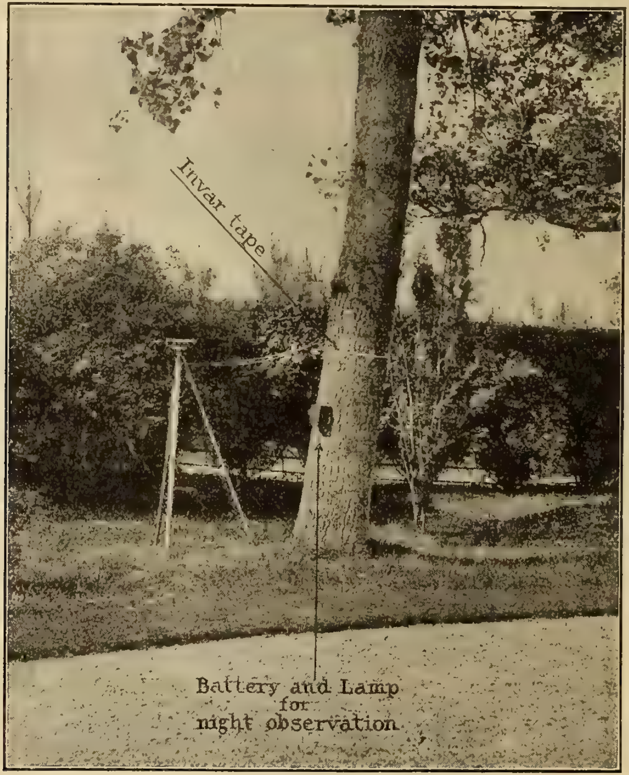

Fig. 1.

The arrangement for using these bands in the measurement of the growth of trees is shown in figs. 1 and 2. At the place of measurement (usually about 5 feet above the ground) a tape of "invar" is passed round the trunk, the roughnesses of the bark having been previously smoothed with a rasp.

The tape is passed over the rockers R_1, R_2, as in fig. 2,* and is kept in constant tension by the spiral springs S_1, S_2, one of which is hooked on to a ring at the end of the tape, and the other to an adjustable clamp, gripping the tape at an appropriate place. To each rocker an arm is attached, carrying a cylindrical stud, H_1, H_2. Thus any expansion or contraction in the girth of the tree causes the distance between the studs to increase or diminish, the friction between the tape and rockers under the tension of the springs being quite sufficient to prevent slipping.

The optical part of the apparatus (figs. 2 and 3) hangs freely from an arm A, projecting from the stout plate on which the rockers are mounted. The plate itself is attached to the tree trunk by screws. P is a right-angled glass prism mounted on a support C, capable of turning about the axis D in the outer frame B, and the angular position of the prism, with reference to B, can be adjusted by the micrometer screw G. Q is a flat glass plate, blacked on the hind surface, and having a straight edge at F. The plate is

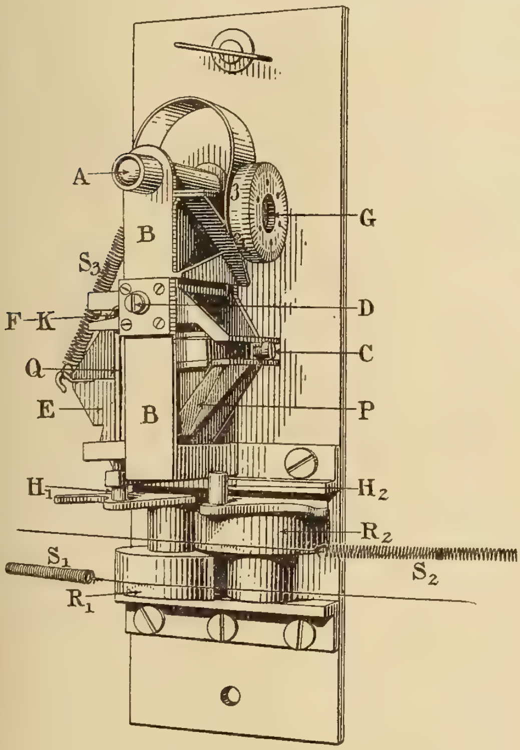

Fig. 2.

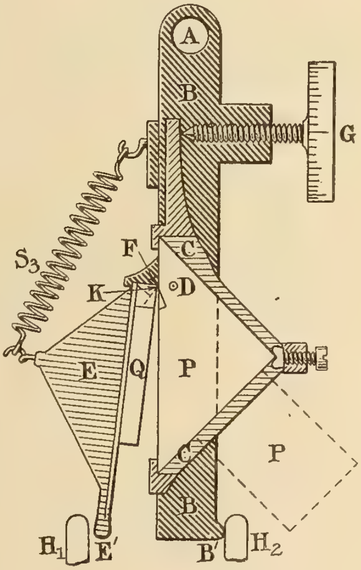

Fig. 3.

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* In fig. 2 the tape is replaced by a thread, so that the rockers may not be hidden from view.

mounted on the stiff support E, and the edge F is kept pressed against the face of the prism by a light spring S_3, which also tends to turn the plate about the edge (and the knife-edges K in line with it) outward from the face of the prism. The distance between E′ and B′ (fig. 3), and therefore the angle between the plate and prism, is limited and defined by the two studs on the rockers, thus any change in the girth of the tree causes a corresponding change in the angle between the two glass surfaces.

When preparing for a set of observations, the bedplate is first secured to the tree, and the "invar" tape is then passed round the smoothed track on the bark and over the rockers, and the tension springs are hooked on to the tape and secured. The prism holder is next put in place, and the two rockers are turned until the studs bring the surfaces of the plate and prism into contact. The interference bands are now very broad (in fact, if the surfaces were truly flat and truly in contact, the field would appear of one uniform shade). The micrometer screw is now turned until the bands assume a width convenient for observation.

In order to define the position of the bands, a narrow central streak M (fig. 4) is painted on the face of the prism with an alcoholic solution of safranin or other suitable anilin colour. This, when dry, is only a small fraction of a wave-length in thickness, but the colour is quite apparent by transmitted light. A narrow gap T is made in the streak by means of a pointed piece of hard wood wetted with alcohol, and this serves as a mark to which the position of the interference bands can be referred. When very small variations of the girth of the tree, such as may occur every few minutes, are to be observed, it is convenient to measure them by estimating the fraction of the band which crosses the gap, but, for ordinary work, where the intervals between the observations are an hour or more, the plan adopted is to bring back the bands to their previous position by turning the micrometer screw, whose readings give directly the variation of angle between the plate and prism which has taken place since the previous observation. Here the optical theory does not enter, and the bands are simply used as delicate callipers.

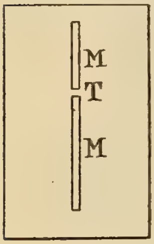

Fig. 4.

To facilitate observation, a second right-angled prism (P′, fig. 3) is cemented to the interference prism so as to reflect the emerging pencils in a horizontal direction to the small telescope, seen in fig. 1, whose aperture is reduced sufficiently to give good definition to the bands.

The first trials of the apparatus were made in April and May, 1917, at Kew, where, by the kindness of Sir David Prain, I was enabled to make observations on several species of trees. It was soon found that the rate of growth was different in each case, but always greater in the early part of the day than later. In fact, actual contraction was noticed on several occasions between noon and 3 P.M. This showed that there was a considerable daily component involved, but its magnitude could not be determined with any certainty by observations which began at 10 A.M. and ended at 5 P.M.

From June 21 to the end of July, while staying in the country, I made constant observations day and night on four trees, devoting a week or ten days to each. The records so obtained are reproduced in Diagrams I–IV, together with the temperature of the air.

It will be noticed that the increase of girth and the temperature curve are rather closely related, the growth being most rapid when the temperature is lowest, or nearly so. Also that rain has a great effect, any shower being followed by increase of girth.

A probable explanation of these facts may be found in the variable rate of evaporation from the leaves, combined with a nearly constant flow of sap into the roots. Presumably, the mean line through the diagram of girth indicates the rate of formation of new wood, the divergence from the mean representing the degree of turgescence in the bark and layers immediately underlying it.

The effect of rain may be partly mechanical, that is, it may act by merely wetting the bark and thus causing it to swell; at any rate, this may happen in the case of heavy rain, but it must also act by checking evaporation from the leaves, and in the case of light showers this is probably the most efficient factor.

I regret that I had no means of measuring the humidity of the air. In any future trials this should be observed. The daily component, as well as the average growth, varies largely in the different trees, and far more extended observations would be required before any generalisation should be attempted.

The method of measurement, however, is simple and satisfactory, and the results, as far as they go, seem sufficiently interesting to warrant their publication.

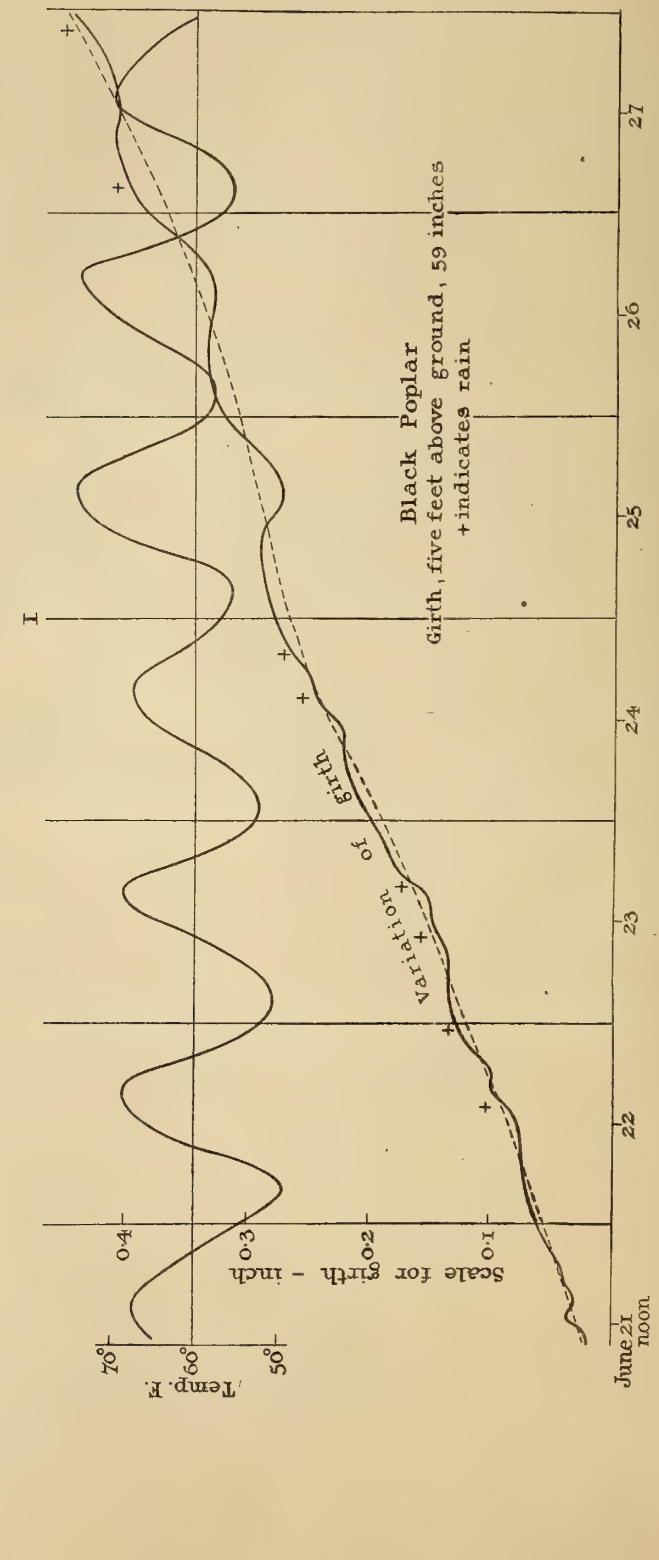
Temp. F.
70°
60°
50°
0·4
0·3
0·2
0·1
Scale for girth – inch
June 21 noon
22
23
24
25
26
27
I
variation of girth
Black Poplar
Girth, five feet above ground, 59 inches
+ indicates rain

Diagram I.

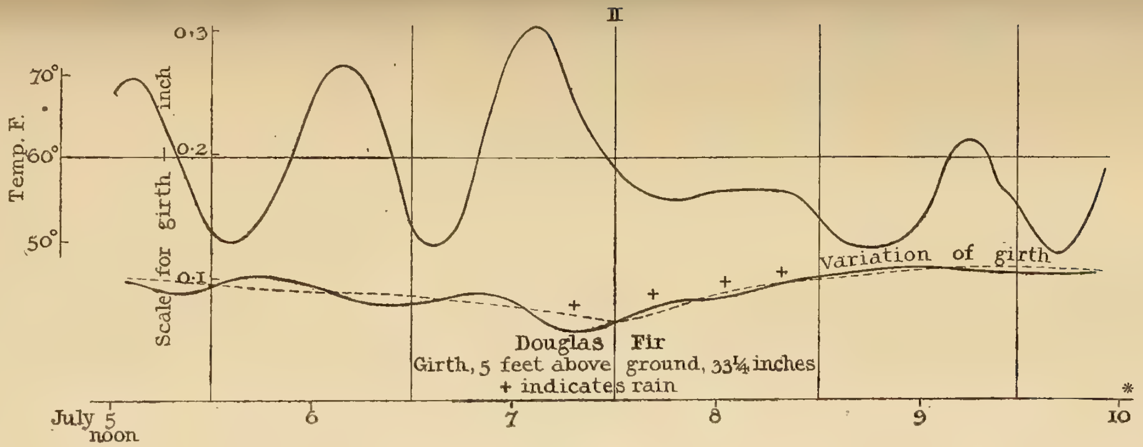

Diagram II.

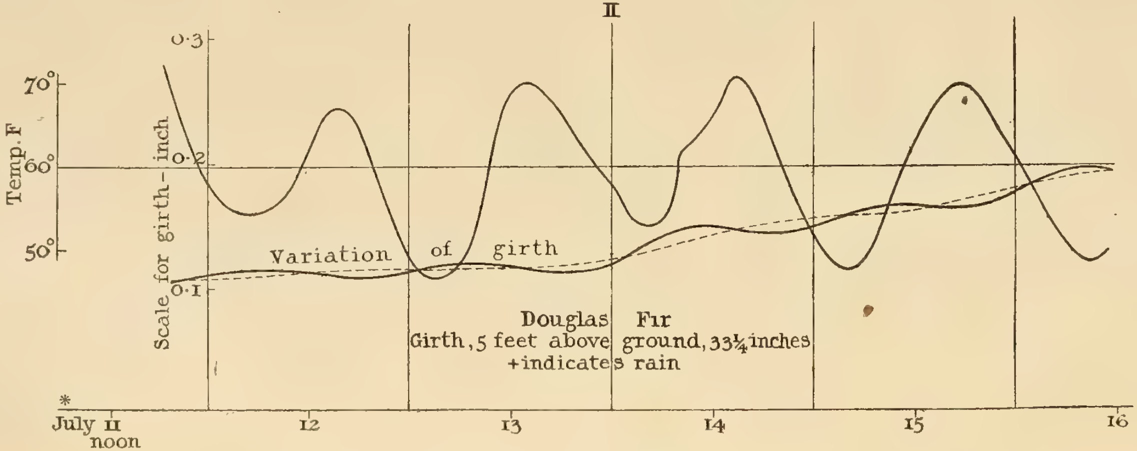

Diagram II—*continued.*

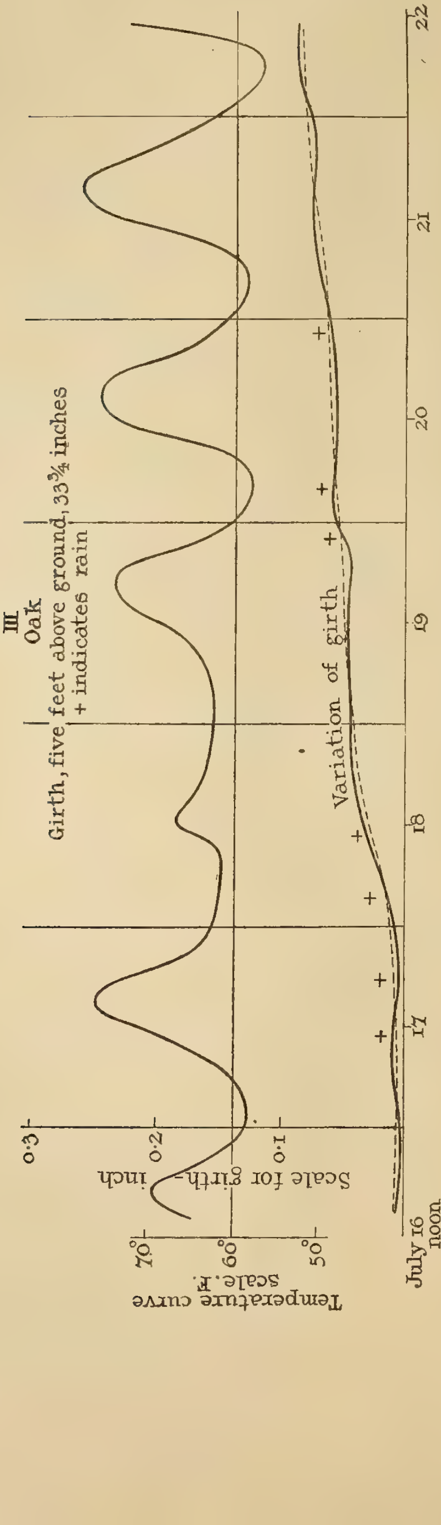
III
Oak
Girth, five feet above ground, 33¾ inches
+ indicates rain
Variation of girth
Temperature curve scale, F.
70°
60°
50°
Scale for girth—inch
0·3
0·2
0·1
July 16 noon
17
18
19
20
21
22

Diagram III.

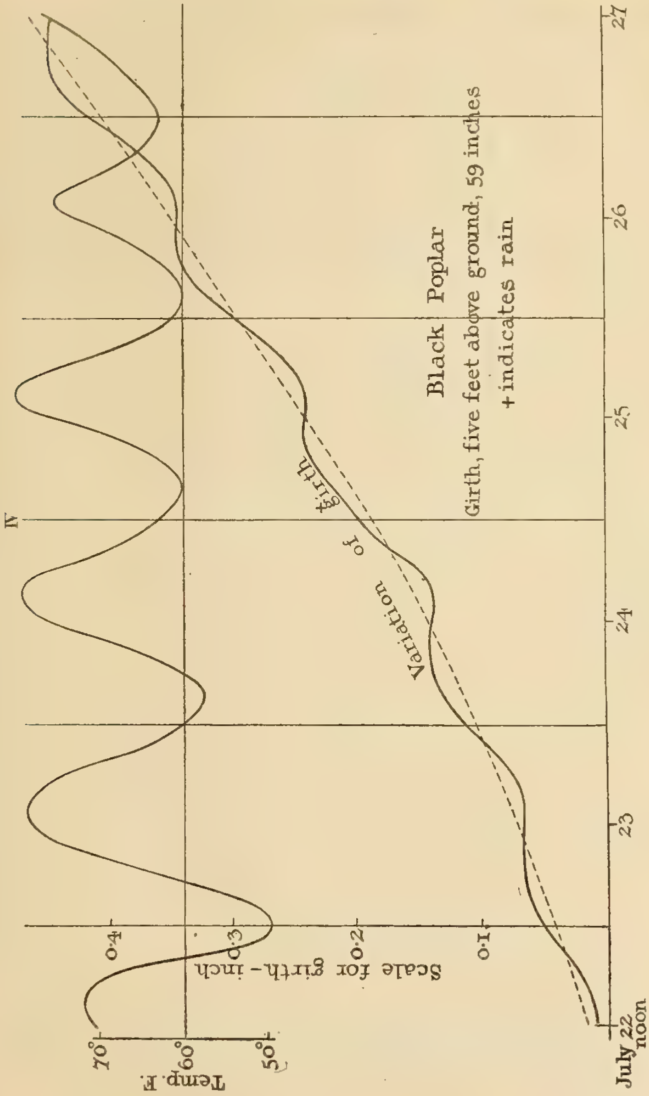

Diagram IV.

Note on Interference Bands Formed by Rays Making Small Angles with the Reflecting Surface.

When light falls very obliquely on flat superposed plates separated by a small interval, no interference bands can be distinguished, owing to the nearly complete reflection which takes place at the first surface and overpowers the weak rays penetrating to, and reflected from, the surfaces where the interference occurs. If, however, a prism is substituted for the upper plate, the interference bands, due to rays whose path in the air space between the surfaces makes a small angle with the surfaces themselves, can be easily observed.

When the angle of emergence (i) from the face of the prism is nearly $\pi/2$, and the angle (r) at which the ray strikes the face internally is therefore, nearly rn^{-1}/μ, a small change in r causes a large change in i.

If $i = 90-\gamma$, so that γ is the angle which the emergent ray makes with the face of the prism, and if e is the difference between r and the angle of total internal reflection (r_0), then $dy/de = (\mathrm{B}+e)/\sqrt{(2\mathrm{B}e)}$, where B stands for $\mu \cos r_0$.

Thus, when $e = 0$, $dy/de = \infty$. This has an important effect on the positions and appearance of the interference bands.

Below the face of the prism (fig. 5) let there be a flat glass plate touching the face at O, and inclined to it at a very small angle α. Take O as the origin, and the face of the prism as containing the axis of x; then the distance y between the two surfaces at x is αx.

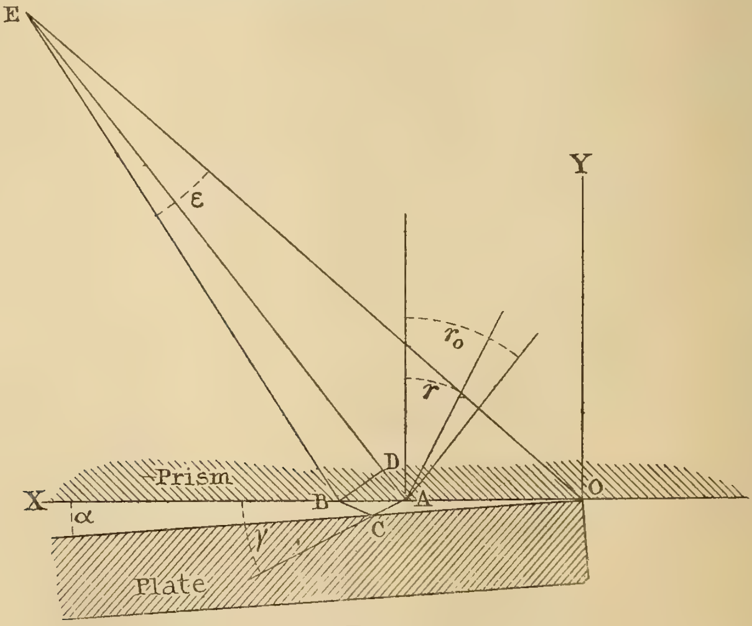

Fig. 5.

If a ray within the prism strikes the surface at A, making an angle r with the axis of y, the transmitted part makes an angle γ with the face, and, being reflected from the lower plate, again enters the prism in a direction hardly differing from r (on account of the smallness of α). It is then in a condition to interfere with the ray reflected from the prism surface at its point of entry. The optical lengths of the paths of the interfering rays are respectively $AC+CB$ in air, and μAD in glass. The difference in their length is easily shown to be equal to $2y\gamma$.

Let the surfaces be viewed from a point E at a distance L from X; then, noting that $OEB = e$, and writing B for $\mu \cos r_0$, it will be found that $Y = \sqrt{(2X/\mu L)}$, and that, consequently, the relative retardation of the interfering rays is $2aBX\sqrt{(2x/\mu L)}$.

Taking into account the half-wave-length change of phase at the internal reflection at B, the bands are bright or dark, according as the retardation is $n\lambda$ or $\frac{1}{2}(2n+1)\lambda$.

Hence for the bright bands $$x = \left(\frac{n\lambda}{2aB}\right)^{\frac{2}{3}} (\mu L)^{\frac{1}{3}}$$

for the dark bands $$x = \left(\frac{2n+1}{4aB}\lambda\right)^{\frac{2}{3}} (\mu L)^{\frac{1}{3}}$$

so that their spacing is not uniform.

These bands differ in several other ways from those formed by normal, or nearly normal, incidence. The latter practically have an objective existence at the surfaces of interference, and can be viewed by a telescope adjusted to focus an object at the distance of the plate from the observer, the reason being that the size and position of the bands change only slowly with the angle of incidence. In the case at present under consideration this condition does not hold. As Stokes* puts it:—

> "When the angle of incidence becomes nearly equal to that of total reflection, a small change of obliquity produces a great change in the order of the ring to which the reflected ray belongs, and therefore the rings are indistinct to an eye adapted to distinct vision of the surface of the glass. They are also indistinct, for the same reason as before, if the eye be adapted to distinct vision of distant objects. To see distinctly the rings in the neighbourhood of the angle of total internal reflection, the author used a piece of blackened paper, in which a small hole was pierced with the point of a needle."

Stokes is here speaking of Newton's rings. In the case of bands between flat plates, the aperture of the pupil need not be limited, if the observation be made at a distance of some feet.

Another peculiarity of these bands may be mentioned. When formed by white light, the bands are, of course, coloured. For those distant from the line of total reflection the blue is on the inside, *i.e.*, nearest to that line, but, for the band close to the line, the blue is on the outside. Between them occurs a band which appears achromatic. The explanation is shortly as follows: As has been stated, the relative retardation of the interfering rays is $2y\gamma$. Here γ (and in a less degree y) varies with μ, and with the wave-length. If two rays, say, yellow and blue, are to reach the eye in the same direction and with the same relative retardation, then must $y_Y\gamma_Y/\lambda_Y = y_B\gamma_B/\lambda_B$. The difference between y_Y and y_B is small compared to the difference between γ_Y and γ_B, and therefore there is approximate achromatism when $\gamma_Y/\gamma_B = \lambda_Y/\lambda_B$.

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* Stokes, 'Collected Papers,' vol. 2, p. 359, from 'B.A. Report,' 1850.

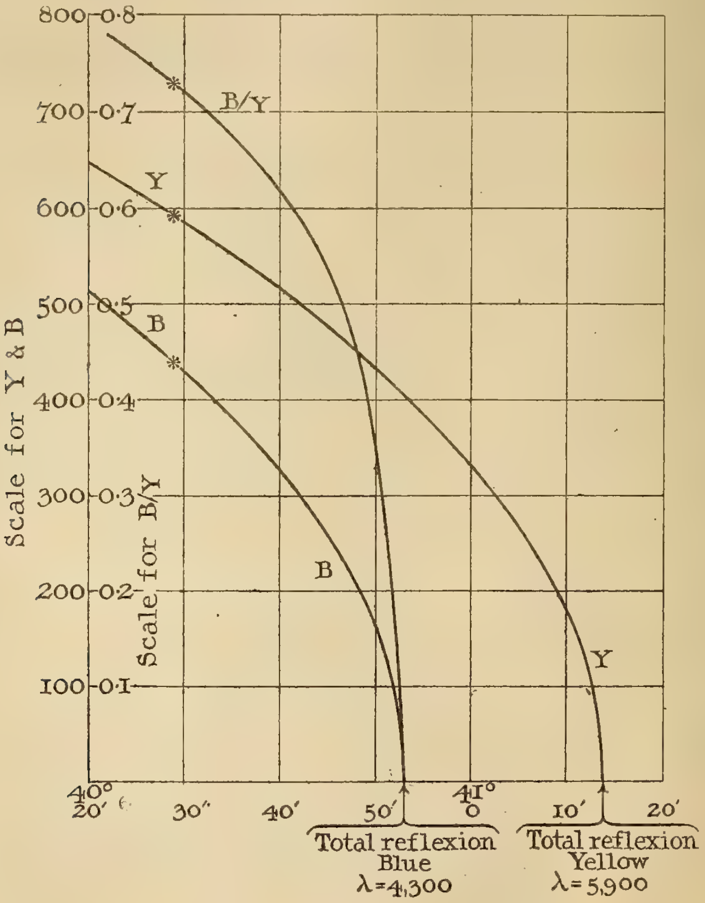

FIG. 6.

The diagram (fig. 6) will make this clearer. The two curves Y and B (which relate to a sample of crown-glass) give the value of γ in terms of r, in the neighbourhood of the angle of total internal reflection, Y referring to the yellow of the D line, and B to the blue near G, viz., at $\lambda_Y = 5900$ and $\lambda_B = 4300$.

It will be seen that there is one value for r (marked by * on the figure) and one only, which makes $\gamma_B/\gamma_Y = \lambda_B/\lambda_Y = 0{\cdot}73$, viz., when $r = 40^\circ\ 29'$ (45′ less than the angle of total reflection for the yellow and 24′ less than for the blue).

At the position so defined there will be a band with borders only faintly coloured. On either side of this band the order in which the colours appear will be reversed.

On the Efficiency of Muscular Work.

By M. Greenwood, Captain R.A.M.C. (T.F.), Lister Institute of Preventive Medicine.

(Communicated by Prof. Leonard Hill, F.R.S. Received January 18, 1918.)

In a paper communicated to the Royal Society in 1913,[*] Prof. J. S. Macdonald published a series of observations upon the heat production of persons performing certain known quantities of work upon a bicycle ergometer. In that paper, and again in a more recent publication,[†] Prof. Macdonald has outlined certain methods of interpreting his results, which are of much importance; these I shall discuss in the latter half of this communication, but, before doing so, it will be interesting to examine some purely numerical questions to which Macdonald's paper gave rise.

In a note on Macdonald's earlier paper, Messrs. Glazebrook and Dye[‡] have published a formula descriptive of Macdonald's numerical results. This formula is

$$H = a + bM + \frac{W}{\alpha + \beta M}, \tag{1}$$

where H = heat production in calories, M = body mass in kilogrammes, W = work equivalent in calories, a, b, α, and β are constants.

The values of the constants, which were obtained by a graphical process led in the particular case to the equation

$$H = -138 + 4{\cdot}5M + \frac{W}{0{\cdot}08 + 0{\cdot}003M}, \tag{2}$$

and this equation was found to provide values in very fair agreement with the observed results.

It will be noticed that, when the body weight is constant, the heat production is, by Glazebrook and Dye's formula, a linear function of the external work performed. For a constant performance of work, the change of heat production with changing mass is described by a hyperbola, one asymptote of which is parallel to the axis of H (for $W = 0$, the equation is that of the asymptotes), and, in consequence of the position of the principal axes with reference to the axes of H and M, the minimum of H moves to the right with increasing values of W.

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[*] 'Roy. Soc. Proc.,' B, vol. 87, p. 96 (1914).

[†] *Ibid.*, vol. 89, p. 394 (1917).

[‡] *Ibid.*, vol. 87, p. 311 (1914).

In effect, we have

$$\frac{dH}{dM} = b - \frac{W\beta}{(\alpha+\beta M)^2}, \tag{3}$$

$$\frac{d^2H}{dM^2} = \frac{2\beta^2 W}{(\alpha+\beta M)^3}, \tag{4}$$

(4) is always positive and from (3) the value of M for a minimum is

$$M = -\alpha/\beta \pm \sqrt{(W/b\beta)}.$$

This decreasing disadvantage, or, rather, increasing advantage, of mass with increase of work is in agreement with the remarks of Prof. Macdonald,* and, *pro tanto,* is an argument in favour of the form of Glazebrook and Dye's expression. But that the formula itself is no more than an interpolation formula is manifest, since it gives negative values of H for small values of M when $W = 0$, while, even within practicable ranges, it leads to the paradoxical result that the absolute heat production associated with the performance of 56 thermal units of work in a mass of 20–30 kgrm. is not less than that of a mass between 50 and 60 kgrm. I thought, therefore, that it would be of interest to determine whether a linear interpolation formula might not reproduce the experimental results with an accuracy comparable with that achieved by Glazebrook and Dye's second degree expression. Taking the data on p. 313 of Glazebrook and Dye's paper, I computed the various constants of a first degree multiple regression equation; these are set out in Table I, and the deduced equation is

$$H = 24{\cdot}900 + 3{\cdot}940\,W + 1{\cdot}755\,M. \tag{5}$$

Testing this against the observations, I obtain Table II, which is a reproduction of Glazebrook and Dye's Table III, with the addition of the values computed from the above regression equation. It is easy to see that the linear expression is not inferior to Glazebrook and Dye's expression in its power to reproduce the experimental results, although it has one less constant (actually, the mean square of error is rather smaller in the present case, but this is largely because of the failure of Glazebrook and Dye's formula in connection with the individual weighing 43·7 kgrm., and, in any event, the test is a crude one, so that no claim is made that the regression formula is the better of the two). The regression equation does not lead to any paradoxical negative values of heat production, but that it also is only an interpolation formula is seen in the circumstance that the computed heat production at rest exceeds the experimentally determined values of the heat production in sedentary occupations, and, *a fortiori*, those of persons resting in bed.

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* *Op. cit.*, p. 111.

The result, however, suggested the desirability of examining in the same way other data.

Table I.—Constants Deduced from Data of Glazebrook and Dye's Table II (*op. cit.*, p. 313).

	Heat production.	Work.	Mass.
Mean	260·042	34·042	57·542
Standard deviation ...	64·515	16·016	6·452

$r_{HW} = 0 \cdot 9735$ $\quad {}_{M}r_{HW} = 0 \cdot 9889$ $\quad {}_{WM}\Sigma_{H} = 9 \cdot 478$

$r_{HM} = 0 \cdot 1493$ $\quad {}_{W}r_{HM} = 0 \cdot 7668$ $\quad {}_{HM}\Sigma_{W} = 2 \cdot 379$

$r_{WM} = -0 \cdot 0269$ $\quad {}_{H}r_{WM} = -0 \cdot 7608$ $\quad {}_{HW}\Sigma_{M} = 4 \cdot 140$

For this purpose I selected the observations of Amar.* This investigator also made use of a stationary bicycle as an instrument for measuring work performance. The subjects of the experiment were Algerians accustomed to heavy manual labour, and the period of trial several hours. The determinations of heat production were much cruder than those of Macdonald, and depend upon analyses of the ingredients of a diet sufficient to maintain the subject in equilibrium (it would seem that constancy of weight was all that was attempted to be maintained, separate balance sheets of the factors involved not being, from the nature of the experiments, capable of preparation) or upon the oxygen use. The heat productions determined by the two methods did not agree at all closely, and I have followed the author in paying special attention to the heat values of the equilibrium diets. Table III reproduces the observations, the amounts of work having been converted from kilogramme-metres to thermal units.

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* 'Le Rendement de la Machine humaine,' Jules Amar, Paris, 1910.

Table II.—Comparison of Observed and Calculated Heat Production.

Work per hour in calories	0			13			19			43			56		
Body mass in kgrm.	Heat production.			Heat production.			Heat production.			Heat production.			Heat production.		
	Obs.	Calc. I.	Calc. II.	Obs.	Calc. I.	Calc. II.	Obs.	Calc. I.	Calc. II.	Obs.	Calc. I.	Calc. II.	Obs.	Calc. I.	Calc. II.
43·7	—	59	102	—	—	—	177	149	176	279	263	271	346	319	322
54·6	—	108	121	160	161	172	193	186	196	280	284	290	335	338	341
55·7	—	113	123	169	166	174	—	—	—	285	287	292	—	—	—
58·8	—	127	128	181	178	179	—	—	—	298	295	298	—	—	—
60·5	—	134	131	—	—	—	212	206	206	317	299	300	347	348	352
61·9	—	141	134	186	190	185	216	212	208	306	303	303	348	352	354
66·7	—	162	142	209	208	193	—	—	—	324	315	311	352	362	363

Table III.—Observations of Amar.

Weight.	Work.	Heat production.	Weight.	Work.	Heat production.
kgrm.	calories.	calories.	kgrm.	calories.	calories.
76·2	156·8	3398	64·8	137·5	3020
71·3	114·1	2988	60·2	129·7	2812
69·6	142·6	3048	72·4	97·1	2962
58·0	142·6	2781	68·9	129·4	3236
74·6	142·6	2912	70·1	129·4	3214
68·9	128·3	3135	70·8	161·7	3389
69·1	142·6	3261	66·5	129·4	2908
62·1	156·8	3030	66·7	137·5	3063
68·7	128·3	3139	71·2	129·4	2956
65·4	142·6	2996	72·4	145·6	3023
70·4	128·3	3248	69·3	129·4	3001
69·1	142·6	3117	67·4	145·6	2841
63·7	142·6	2891	69·6	161·7	3117
62·1	114·1	2667	66·2	121·3	2733
73·5	142·6	3403	74·5	121·3	2808
61·3	129·4	2999	67·7	97·1	2813
70·1	137·5	3318	57·5	97·1	2615
79·8	121·3	2989	70·4	113·2	2814
61·3	129·4	3936			

The statistical constants are shown in Table IV, and the deduced equation is

$$H = 6{\cdot}244W + 17{\cdot}777M + 977{\cdot}5. \qquad (6)$$

Table IV.—Constants Deduced from Table III.

	Heat production.	Work.	Mass.
Mean	3015·70	132·392	68·157
Standard deviation	197·927	15·968	4·917

$r_{HW} = 0{\cdot}5469$ $\qquad {}_{M}r_{HW} = 0{\cdot}5754$ $\qquad {}_{WM}\Sigma_{H} = 141{\cdot}039$

$r_{HM} = 0{\cdot}4909$ $\qquad {}_{W}r_{HM} = 0{\cdot}5250$ $\qquad {}_{HM}\Sigma_{W} = 12{\cdot}997$

$r_{WM} = 0{\cdot}0977$ $\qquad {}_{H}r_{WM} = -0{\cdot}2340$ $\qquad {}_{HW}\Sigma_{M} = 4{\cdot}165.$

Table V compares the computed with the observed values. The agreement between the computed and the observed values is reasonably close, while, as seen in Table VI, the distribution of deviations, expressed in terms of the standard deviation of the prediction, is not an improbable one. The data just analysed differ in kind from those of Macdonald, since there is but one observation with respect to each individual, instead of a number of observations upon each of a few individuals. Again, the heat production is that of the whole day, so that the estimated efficiency is a mere average. But such a sample is of interest from the industrial standpoint, in so far as it throws light upon the question of the ration needed for varying amounts of

Table V.

Observed heat production.	Calculated heat production by equation (6).	Difference.	$\frac{\text{Difference}}{\text{WM}\,\Sigma_{H}}$.	Difference as percentage of observed value.
3398	3311	− 87	−0·62	2·6
2988	2957	− 31	−0·22	1·0
3048	3105	+ 57	+0·40	1·9
2781	2899	+118	+0·84	4·2
2912	3194	+282	+1·999	9·7
3135	3003	−132	−0·94	4·2
3261	3096	−165	−1·17	5·1
3030	3060	+ 30	+0·21	1·0
3139	3000	−139	−0·99	4·4
2996	3030	+ 34	+0·24	1·1
3248	3030	−218	−1·55	6·7
3117	3093	− 24	−0·17	0·8
2891	3000	+109	+0·77	3·8
2667	2794	+127	+0·90	4·8
3403	3174	−229	−1·62	6·7
2999	2875	−124	−0·87	4·1
3318	3082	−236	−1·67	7·1
2989	3153	+164	+1·16	5·5
2936	2875	− 61	−0·43	2·1
3020	2988	− 32	−0·23	1·1
2812	2858	+ 46	+0·33	1·6
2962	2871	− 91	−0·65	3·1
3236	3010	−226	−1·60	7·0
3214	3032	−182	−1·29	5·7
3389	3246	−143	−1·01	4·2
2908	2968	+ 60	+0·43	2·1
3063	3022	− 41	−0·29	1·3
2956	3051	+ 95	+0·67	3·2
3023	3174	+151	+1·07	5·0
3001	3017	+ 16	+0·11	0·5
2841	3085	+244	+1·73	8·6
3117	3224	+107	+0·76	3·4
2733	2912	+179	+1·27	6·5
2808	3059	+251	+1·78	8·9
2813	2787	− 26	−0·18	0·9
2615	2606	− 9	−0·06	0·3
2814	2936	+122	+0·87	4·3

Table VI.—Distribution of Deviations.

	Observed.	On the basis of a "normal" curve.
Greater than the standard deviation, positive	6	5·87
Between 0 and „ „ „	12	12·63
„ „ „ „ negative	12	12 63
Greater than „ „ „	7	5·87

muscular work. The particular work here chosen was abnormal in kind rather than in degree. In his recent treatise,* Amar has estimated the work of a metal filer in an 8½ hours' day to be 61,600 kilogramme-metres; that of a smith as rather more than twice as much. Taking these quantities as equivalent to a medium and a heavy day's work, it will be interesting to compute from equation (6) the requisite energy requirements of a man weighing 67 kgrm. Taking the round numbers 60,000 and 120,000 kilogramme-metres, and reducing to thermal equivalents, we reach 3049 calories for the medium and 3929 calories for the heavy work.

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* Amar, 'Le Moteur Humain,' p. 527 and p. 547, Paris, 1914.

These figures are not very different from the usual standards. So far I have merely examined the data from the same standpoint as that of Glazebrook and Dye, viz., that of finding a formula which shall summarise particular experimental data, and it appears that this comparatively humble task can be readily accomplished, *i.e.*, we can, with very little arithmetical work, construct interpolation formulæ which reproduce the observations sufficiently well for the formulæ to be used to approximate to intermediate values within the range of the data. It is, however, more than doubtful whether such formulæ throw light upon the efficiency of the body as an energy transformer when this term is strictly used, and I now propose to examine the fundamental problem.

The customary method of determining the efficiency of the human body as a machine, prior to the publication of Macdonald's paper, was to subtract from the total heat production associated with the performance of a measured amount of external work the heat production of the same subject when "at rest"; the heat equivalent of the work divided by this has been termed the "net efficiency," the "gross efficiency" being the quotient of work by total heat production. Clearly the "net efficiency" will vary with the base line chosen, *i.e.*, whether "at rest" is taken to be lying on a couch, sitting still on the ergometer, rotating its pedals without load, etc. Benedict and Cathcart* discuss the choice of base line at length in their admirable monograph, and point out the inconvenient diversity of base lines used or proposed. But even these writers do not remark that the method is fundamentally open to attack. Thus, if heat transformed be a function of work, the proper measure of extrinsic efficiency would seem to be the reciprocal of the first derivative of H with respect to W, the limiting value of the incremental change of heat with work. This measure will differ considerably in value from the "net efficiency" and may even lead to a different interpretation of the results. For instance, Benedict and Cathcart† note a tendency towards an increase in both gross and net efficiencies with increasing loads. With

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* Benedict and Cathcart, 'Muscular Work: a Metabolic Study, with Special Reference to the Efficiency of the Human Body as a Machine,' Washington, 1913.

† *Op. cit.*, p. 125.

0·5 ampère load their professional subject had an efficiency of between 15 and 18 per cent. (revolution rate between 67 and 72), which rose to 24 per cent. when the load was furnished by a 1·5 ampère current. The reader might infer that the muscular efficiency improved with load. This, however, is a mere consequence of the method of calculation. Table VII gives the observations, and it will be seen that for this range the linear equation $H = aW + c$ very fairly represents the data. Adopting the above definition, the efficiency is $1/a$, in the particular case, 0·2993. Now Benedict and Cathcart's subject's net efficiency is $W/(aW + c - 1{\cdot}17)$ and his gross efficiency $W/(aW + c)$, which obviously increase with W and will only reach the value $1/a$ when W is infinite. In effect, using the formula we have, for the particular values 0·48 cal. and 1·57 cal., "net efficiencies" 0·17 and 0·24; for "gross efficiencies," 0·12 and 0·21, which agree, to this number of decimals, with the observed means.

Table VII.—Heat Production and Work. Observations of Benedict and Cathcart on M. A. M. pedalling at the rate of 68–72 revolutions per minute (inclusive).

Work done.	Observed heat production.	Mean of observations.	Heat production given by formula Heat = 3·3415 Work + 2·4131.
calories.	calories.		
0·47	4·01, 4·01	4·01	3·98
0·48	3·86, 3·88, 3·83, 4·13, 3·96	3·93	4·02
0·49	4·15, 3·94	4·05	4·05
1·19	6·73	6·73	6·39
1·20	6·65, 6·81	6·73	6·42
1·33	6·95	6·95	6·86
1·35	6·97	6·97	6·92
1·36	7·08	7·08	6·96
1·56	7·44, 7·66	7·55	7·62
1·57	7·87, 7·59, 7·53, 7·64, 7·41, 7·51, 7·55	7·59	7·66
1·59	7·56	7·56	7·73

Hence the data are quite consistent with a constant efficiency as just defined for all loads within the observed range. *A fortiori*, Amar's base line is highly objectionable, and no doubt over-estimates the real efficiency of the machine. A labourer who rests on Sunday and does eight hours' work on Monday really performs some muscular work, although not industrial work, on Sunday; the Monday's work is not a mere addition to that of Sunday, but, in part, a substitution of purposeful for aimless activity. Hence to deduct the whole of the heat production on the rest day from the working day's quota is to rate the efficiency too high.*

Macdonald's recent paper is an attempt to transfer the inquiry to a different plane altogether. If I have followed his extremely ingenious reasoning correctly, his position is as follows. He holds that the efficiency of work performance is a function of body mass, and he found that when the thermal equivalent of the external work performed was divided by the efficiency (as defined above) and the quotient subtracted from the total heat production, the residue was, in his experiments, sensibly constant for a given range of work performance. This residue is defined as the cost of movement, it varies with the velocity of movement and can again be expressed as a function of mass and rate of movement.

The details of Macdonald's analysis are perhaps open to criticism. Thus the agreement between his arithmetical calculations and the observed results is not always of such importance as appears on the face of the figures, since he is using formulæ with several constants for absolutely few observations.

Thus, he has a theoretical formula for cost of movement (Q) in terms of velocity of movement, an expression of the form $Q = a(bV)^{cV}$, where a, b and c are constants to be determined from five observations only. But these criticisms are of subsidiary importance and detract in no way from the suggestiveness of the argument. Looking at the theory as a whole, the following conclusions appear to follow. The real efficiency of the muscular machine is indeterminate and may even be unity. The apparent efficiency of the same individual performing the same kind of work at different rates will be represented by the reciprocal of the first derivative with respect to W of $H = aW + b$, *i.e.*, is equal to $1/a$; a is constant and $1/a$ might be called the indicated efficiency, but b (the cost of movement) will vary with the rate of performance, being a minimum at the most economical rate of movement. Thus the locus of heat produced as a function of work is a family of parallel straight lines intersecting the axis of heat at different points.†

It seemed desirable to test the deduction on the basis of Benedict and Cathcart's important data. To this end the observations upon their professional subject were sorted out into groups for each of which the rate of pedalling fell within narrow limits. Only two such groups were really suitable for the purpose; that already given in which the rate of pedalling was between 68 and 72 and the group 98 to 102. In the remainder either the number of observations was less than 20 or the range of variation of work performed very small. In Table VIII are reproduced the second available set. The linear equations are

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* This criticism is implicit in Lefèvre's excellent discussion of the efficiency problem ('Chaleur Animale et Bioénergétique,' pp. 924, etc., Paris, 1911), which I had not seen until most of this paper had been written.

† It is to be noted that Chauveau's formula also includes a velocity form, see Lefèvre, *op. cit.*, pp. 686 *et seq.*

$$H = 3{\cdot}3415\,W + 3{\cdot}4131 \qquad \text{and} \qquad H = 3{\cdot}61225\,W + 3{\cdot}7543$$

Table VIII.—Heat Production and Work. Observations of Benedict and Cathcart on M. A. M. pedalling at the rate of 98–102 revolutions per minute (inclusive).

Work done.	Observed heat production.	Mean of observations.	Heat production given by formula Heat = 3·61225 Work + 3·7543.
calories.	calories.		
0·55	5·59, 5·28, 6·03, 5·60, 5·64	5·63	5·74
0·56	5·72	5·72	5·78
1·35	8·72	8·72	8·63
1·62	10·15	10·15	9·61
1·63	10·88	10·88	9·64
2·05	11·24	11·24	11·16
2·06	11·30	11·30	11·20
2·07	10·82, 11·21, 11·43, 10·91	11·09	11·23
2·08	11·19	11·19	11·27
2·09	10·98, 11·65, 11·74	11·46	11·30
2·10	11·26	11·26	11·34
2·11	11·13, 11·15, 11·39, 11·35	11·26	11·38
2·12	11·29, 11·35, 11·03, 11·29	11·24	11·41

The first point which arises is whether the observations are, when examined strictly, reasonably probable examples of a linear law (that they are sufficiently so for ordinary purposes of numerical interpolation is no doubt evident). To test the matter, I had recourse to a method proposed by Pearson,* the application of which is seen in Tables IX and X. On the face of the figures, it seems that both cases are very improbable examples of a

Table IX.—Observations of Table VII Grouped for Testing Fit.

Group.	Frequency.	Observed mean.	Calculated mean.	Square of difference.	Square of difference × frequency.
0·47–0·49	9	3·974	4·018	0·0019	0·0172
1·19–1·33	4	6·785	6·523	0·0689	0·2756
1·35–1·56	4	7·288	7·280	0·00006	0·0002
1·57	8	7·583	7·669	0·0074	0·0595
					0·3525

$\sigma_H{}^2(1-\eta^2) = 0{\cdot}01698$; $\chi^2 = 20{\cdot}760$; P = 0·00036.

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* 'Biometrika,' vol. 11, p. 237 (1916).

Table X.—Observations of Table VIII Grouped for Testing Fit.

Group.	Frequency.	Observed mean.	Calculated mean.	Square of difference.	Square of difference × frequency.
0·55	6	5·643	5·747	0·0108	0·0648
1·35	1	8·720	8·631	0·0079	0·0079
1·62–1·63	2	10·515	9·625	0·7921	1·5842
2·05–2·10	11	11·248	11·254	0·00004	0·0004
2·11	4	11·255	11·376	0·0146	0·0584
2·12	4	11·240	11·412	0·0296	0·1184
					1·8341

$\sigma_H{}^2 (1-\eta^2) = 0{\cdot}053366$; $\chi^2 = 34{\cdot}368$; $P =$ less than 10^{-4}.

linear law, but some reservations must be made. Thus in each the value of χ^2 is chiefly due to a single group, while, careful as are the experiments, absolute uniformity of external conditions can never be secured in inquiries of this kind, so that too much stress ought not to be put upon absolutely small deviations. I am not confident therefore that the result of this test is sufficient to condemn the law as being inapplicable. I also think that no significance attaches to the difference between the regression coefficients (the reciprocals of the efficiencies). The difference is $0{\cdot}2708 \pm 0{\cdot}0855$, which is more than three times the probable difference, but the applicability of the normal law of error to such cases may justly be questioned; that the divergence may be of no practical importance is suggested by the results obtained with the next higher rates of pedalling, although these are determined from smaller numbers of observations. They are shown in Tables XI and XII, and it will be seen that the regression coefficients do not continue to increase, but are well within the range of likely fluctuation about the value deduced from the observations at lower speeds.

Table XI.—Heat Production and Work. Observations of Benedict and Cathcart on M. A. M. pedalling at the rate of 103–107 revolutions per minute (inclusive).

Work done.	Observed heat production.	Mean of observations.	Heat production given by formula Heat = 3·1262 Work + 5·1525.
calories.	calories.		
0·55	6·01	6·01	6·87
1·35	9·20, 9·78	9·49	9·37
1·64–1·65	11·21, 11·35	11·28	10·30
1·83	11·53	11·53	10·87
2·12–2·17	11·55, 11·63, 11·72, 12·66, 11·33 11·32, 11·83, 11·73, 11·71, 11·23	11·67	11·87

Table XII.—Heat Production and Work. Observations of Benedict and Cathcart on M. A. M. pedalling at the rate of 108–112 revolutions per minute (inclusive).

Work done.	Observed heat production.	Mean of observations.	Heat production given by formula Heat = 3·3271 Work + 5·1980.
calories.	calories.		
0·54–0·55	6·46, 6·70	6·58	7·02
1·37	9·10	9·10	9·76
1·58–1·68	11·29, 11·35, 12·85	11·83	10·64
2·18–2·24	11·77, 13·02, 11·97, 12·72, 12·16 13·08, 11·59, 12·47, 12·17	12·33	12·55

I conclude, then, that the data of Benedict and Cathcart, much the most extensive at our disposal, are consistent with the hypothesis that heat production is, at least to a first approximation, expressible as a linear function of work, $H = aW + b$, a being constant for all observed ranges of work.

A matter which calls for discussion is the economy of thermogenesis in work, a question first, I think, raised by Lefèvre in 1902, and again by Lapicque in 1906.* The discussion of these writers is, although not affected in principle, complicated in detail by their adoption of the glucositic theory of muscular energy, and I shall follow a somewhat different line of thought. The fundamental notion is, of course, quite simple. A certain intensity of pure thermogenesis is necessary for the bioplasm to act as an energy transformer at all; the lower limit of this is given by the heat output at rest. But when the bioplasm performs work, there is, unless the conversion of potential energy into work is complete, an associated liberation of energy as heat and not otherwise available. Hence there is a possibility that some part of the heat production necessary for the existence of the bioplasm might be obtained, so to speak, as a by-product of muscular work. Carrying the point to an extreme, we might have, for a certain range of work performance, the range varying inversely as the real efficiency, merely to introduce equal increments of heat for each increment of work, so that within that range the apparent incremental efficiency, *i.e.*, the indicated efficiency, would be unity, whatever the value of the real efficiency. Of course, in this extreme form, the hypothesis could not possibly be true, for it would involve a complete constancy of heat production (after subtracting from the total heat production the thermal equivalent of the work performed), which, since in work performance the opportunity for heat loss must usually increase, could not happen. But the original idea might, nevertheless, be correct.

---

* Lefèvre, *op. cit.*, pp. 910, etc.

The argument can best be expressed symbolically.

Let h be the resting heat production in unit of time, W the work performed (in thermal units), E′ the real efficiency, *i.e.*, W/E, the energy needed to develop W units of work, and $H' = H - h$. Then we have

$$H' = W/E' - \phi(W)h$$

subject to the conditions

$$\text{(i)}\ \phi(W) \text{ cannot exceed 1 and } \phi(0) = 0,$$

$$\text{(ii)}\ (1-E')(W/E' - \phi(W)h) + h[(1-\phi(W)] = h + \lambda,$$

where λ is an unassigned function of W.

These give
$$\phi(W) = \frac{(1-E')W/E' - \lambda}{(2-E')h},$$

and $\phi(W)$ attains its greatest possible value when

$$W = \frac{E'}{1-E'}\{2-E')h + \lambda\}.$$

So that
$$H = W/E' + h\left\{1 - \frac{(1-E')W/E' - \lambda}{(2-E')h}\right\},$$

until
$$W = \frac{E'}{1-E'}\{(2-E')h + \lambda\},$$

and thereafter
$$H = \frac{W}{E'},$$

hence
$$\frac{1}{E} = \frac{dH}{dW} = \frac{1}{E'} - \frac{(1-E')/E' - d\lambda/dW}{(2-E')} \quad \text{at first,}$$

and subsequently
$$= \frac{1}{E'}.$$

This is, however, inconsistent with the experimental facts, viz., that when W is large, although H is approximately linear in W, it is not equal to aW but to $aW + b$, and arbitrarily assumes λ to be given by $\phi(W)$. Hence we must write our equation in the more general form:—

$$H = W/E' + [1-\phi(W)]h + f(W)$$

subject to
$$\text{(i)}\ \phi(0) = f(0) = 0,$$

$$\text{(ii)}\ W[(1-E')/E'] + [1-\phi(W)]h + f(W) \nless h,$$

$$\text{(iii) At and after } W = k,$$

$$H = W/E' + b, \text{ where } b \text{ is constant.}$$

From (ii) we have
$$\phi(W) \ngtr \frac{f(W)}{h} + \frac{W(1-E')}{E'h},$$

from (iii)
$$h(1-\phi(k)) + f(k) = b,$$

hence, up to W = k,

$$\frac{1}{E} = \frac{dH}{dW} = \frac{1}{E'} + \frac{df(W)}{dW} - \frac{d\phi(W)}{dW} \cdot h,$$

and consequently E might differ considerably from E′, for values of W not exceeding k. But thereafter it will become constant and equal to E′, for $df(W)/dW$ and $d\phi(W)/dW$ will vanish.

The argument may be illustrated on Benedict and Cathcart's cyclist working at rates of 68–72 revolutions per minute. Here, for $W = 0{\cdot}48$ or greater, $H = 3{\cdot}3415\,W + 2{\cdot}4131$. Let us assume that constancy is reached at $W = 0{\cdot}45$, which will be the k of our formula. Hence $E' = 0{\cdot}29926$; putting $h = 1{\cdot}17$ (its mean value in Benedict and Cathcart's series), we have:—

$$1{\cdot}17\,[1 - \phi(0{\cdot}45)] + f(0{\cdot}45) = 2{\cdot}4131.$$

We have no knowledge at all of $\phi(W)$ and $f(W)$ save the initial conditions of each and the terminal value of their sum. Assuming that $\phi(0{\cdot}45) = 1$, and that each is linear for that range, we should have

$\phi(W)$ increasing by 0·222 for each 0·1 calorie for 0 to 0·45 and

$f(W)$ increasing by 0·836.

Hence for values of W up to 0·45, $E = 0{\cdot}1639$; for greater values of W it is equal to 0·2993. The locus of H is formed by two straight lines intersecting at $W = 0{\cdot}45$. Naturally the postulated forms of $\phi(W)$ and $f(W)$ are merely illustrative of the way in which the ultimate approximately linear relation might arise. Generally, the hypothesis is that

$$H = W/E' + F(W),$$

subject to $F(0) = h$ and $F(\propto) = 0$ and the practical condition that $dF(W)/dW$ shall be very small for W greater than a constant value k, say.

There is some formal analogy between this expression and the well-known equation of Chauveau,* and the point arises as to the sense to be attributed to the value $W = 0$.

It is evident that the proposed equation is not even now complete, because the assumption of $E' =$ a constant ignores the non-compensated element of a real thermodynamic transformation (*i.e.*, if in such an incompletely reversible cycle, H′ is the heat of the transformation, and T the absolute temperature, $\int \frac{dH'}{T}$ is not zero). It is, however, convenient to examine the case on the assumption that $W = 0$ corresponds to the point where no external work is being done nor is energy expended in maintaining potential, which would be the case, for instance, when standing erect.

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* See Lefèvre, *op. cit.*, p. 728.

In conclusion, I note certain physiological factors which, coming into play at the commencement of muscular work, can hardly increase in intensity *pari passu* with the output of work, but increase the loss of heat, and therefore depress the apparent efficiency.

The first is the circulatory readjustment, leading to an enhanced flow of blood through the muscles. Evidently this partial diversion of the stream through the viscera has an upper limit which may be reached at a comparatively low rate of muscular output. No measurements suitable for the present inquiry have been made.

A second factor is the loss of heat due to air currents. In such work as that on a bicycle ergometer, currents of some magnitude will be set up by the rotatory movements of the legs. Hill, Griffith and Flack* concluded that the total heat loss from the surface of a wet katathermometer was represented by:—

$$\mathrm{H} = (a+b\mathrm{V}^{1/2})\mathrm{T}+(c+d\mathrm{V}^{1/2})(\mathrm{F}-f)^{4/3}$$

where V is the velocity of air movement, while T, a, b, c, d, F and f are independent of V. The movement factor might help to explain the rise of heat production associated with a rotation of the pedals of the bicycle ergometer by the motor, the subject performing no work, as compared with the man's heat production while sitting still on the machine.

Benedict and Cathcart observed outputs of from 2·22 calories per minute to 4·61 (rates of rotation from 58 to 104) under these conditions. The range for sitting still was from 1·35 to 1·82. This cannot, however, be the whole explanation. The rate of heat loss due to this cause must diminish with V, since, from Hill, Griffith and Flack's equation we have $d\mathrm{H}/d\mathrm{V} = k/\mathrm{V}^{\frac{1}{2}}$, where k is a constant, while Benedict and Cathcart's data show that the rate of increase of heat loss is greater between 86 and 96 revolutions per minute than between 60 and 86. The observations are not, however, very numerous, and I hope to be able to submit in a subsequent paper further experimental results dealing with the point.

The results which emerge from the study here published and seem to me interesting are:—

(1) Within fairly wide ranges, simple formulæ of linear regression describe the relations subsisting between heat production, body mass and work performance with an accuracy sufficient for such purposes as roughly computing the energetic needs of workers, doing the kind of work studied. As, however, this work is of a specially simple kind, the type of calculation is

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* 'Phil. Trans.,' B, vol. 207, pp. 183–220 (1916).

more likely to be useful in connection with military exercises than if applied to industrial labour.

(2) No law connecting heat production and work performance can be properly formulated until the range of experimental observations under uniform conditions has been carried below the starting point of existing records. It is desirable to define muscular efficiency more strictly than has been usual, and it is possible that the results of Macdonald which the present extended analysis of Benedict and Cathcart's data verifies, are open to more than one interpretation.

The Galvanometric Measurement of "Emotive" Physiological Changes.

By A. D. Waller, M.D., F.R.S.

(Received October 26, 1917.)

Object.—The object of these experiments was to determine whether or no in the absence of the ordinary visible signs of emotion (muscular, secretory, etc.), electrical signs of emotive discharges are demonstrable by galvanometer.

Affirmative results reported by previous observers* have not, to my mind, fully established the reality of the ground fact, independently of the slight and ordinarily insensible muscular movements that can be perceived by a thought reader or recorded by suitable apparatus, and, as a first step in the inquiry, I thought it necessary to take simultaneous records of galvanometric and muscular movements.

The following communication deals only with the large and sudden electrical responses that are unmistakeably independent of muscular contraction. The smaller and more gradual fluctuations of more debatable nature will be dealt with in a future communication. For the present state of the subject it is, in my opinion, necessary, in the first instance, to establish as clearly as possible the chief actual facts by actual demonstration.

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* Veraguth, 'Das Psychogalvanische Reflexphenomen," Berlin, 1909. Petersen and Jung, 'Psychophysical Investigations with the Galvanometer and Pneumograph in Normal and Insane Individuals.' Goldsheider, "Der sogenannte psycho-galvanische Reflex und seine physikalischchemische Deutung," 'Pflüger's Archiv,' vol. 162, p. 489 (1915).

For this purpose there were projected upon the screen:—

1. On the upper scale the galvanometer spot, arranged to move to the + side with increased electrical conductivity due to emotional excitement (and returning to the − side with diminishing conductivity accompanying the subsidence of excitement).

2. On the lower scale the shadow of a delicate myograph to show the fluctuations of muscular contraction.

For the purpose of the demonstration, strong (disagreeable) stimuli are used, viz.:—

1. An unexpected loud sound (motor horn).
2. An expected burn (lighted match under hand, the striking of the match being, if possible, utilised as the warning signal).
3. A disagreeable pungent smell, under control of subject.
4. A painful thought, in some degree under control of subject.

Method.—Simultaneous photographic records of the movements and of the electrical resistance (skin) are taken from the extremities (hands and feet) of a subject as quiescent as possible. The subject reclines in an armchair reading an unexciting book, and often becomes somnolent; a stimulus calculated to arouse "emotion" is now made and signalled on the records. The muscle recorder is of sufficient delicacy to show the pulse, and to respond to the slightest unconscious movement. The electrical circuit consists of an accumulator cell (2·5 volts), or of 2 Leclanché cells (2·8 volts), two galvanometers, a resistance (1,000 or 10,000 ohms) that can be put in or out of circuit for the purpose of calibration, and the subject of observation, with unpolarisable electrodes applied to the dorsal and palmar surfaces of the hand or of the foot.

The galvanometric spot of light indicative of current strength wavers under the influence of fluctuations of imbibition, and of contact pressure with slight (unconscious) muscular movement, but also, and quite independently, with altered states of consciousness, especially with such alterations as are sufficiently intense to be attended with subjective, or it may be objective, signs of emotion.

The effect is best demonstrated on the hand or foot; on other parts (forearm, arm, leg, thigh) under similar conditions it is imperceptible. As regards the hand and foot, the palmar surface is effective, the dorsal surface ineffective.

Results.—From these facts a correlation with the presence of sweat glands suggests itself.

But atropin (atropin, four trials by local application of liq. atropiæ, followed by a belladonna plaster for 24 hours) was found not to affect the skin response.

The application of an indiarubber band rendering the limb pulseless and ex-sanguine did not appreciably affect the emotive response.

The most remarkable fact is the response to an idea. In this connection it is further remarkable that the most effective ideas are such as are accompanied by disagreeable or painful emotion. The threat of a burn is often more effective than the burn itself.

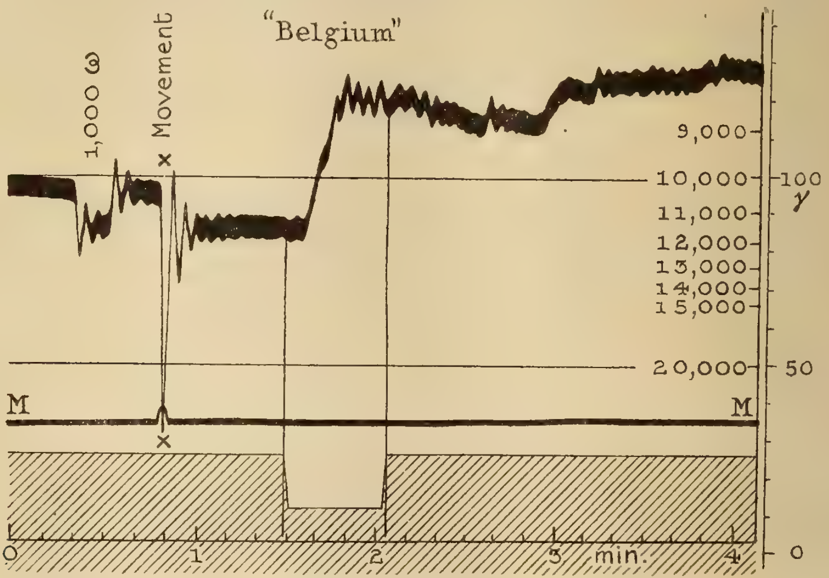

Fig. 1.—The emotive response in this instance has a resistance value of 4000 ohms (*i.e.*, a fall of R from 12,000 to 8000, or a rise of conductivity on the record of about 20 mm. = about 40 γ, *i.e.*, 0·000040 mho).

At the point marked X the subject raised the little finger, causing a rise of the myograph lever and a momentary increase of resistance in the circuit.

The emotive effect of the idea aroused in the subject in response to the sentence "Pensez à la Belgique" was far more permanent than that shown in fig. 2, in which the exciting idea was aroused by the phrase "Pensez aux Gothas." The subject, G. de D., is a Belgian lady, who was an eye-witness of episodes in the German occupation of Belgium in 1914.

Lighting a match may give a larger effect than the application of a match to the free hand.

The apprehension of a bad smell that has just been experienced has proved to be particularly effective.

The expected prick of a needle has produced more effect than an unexpected prick.

PROCEEDINGS
OF
THE ROYAL SOCIETY.

Series B. Vol. 90. **No. B 628.**

BIOLOGICAL SCIENCES.

CONTENTS.

Page

On the Quantitative Differences in the Water-Conductivity of the Wood in Trees and Shrubs. Part I.—The Evergreens. By J. BRETLAND FARMER, F.R.S., Professor of Botany in the Imperial College of Science and Technology **218**

On the Quantitative Differences in the Water-Conductivity of the Wood in Trees and Shrubs. Part II.—The Deciduous Plants. By J. BRETLAND FARMER, F.R.S., Professor of Botany in the Imperial College of Science and Technology **232**

The Production of Anthocyanins and Anthocyanidins.—Part III. By ARTHUR ERNEST EVEREST, D.Sc., Ph.D. **251**

Note on the Nature of Growths in Colloidal Silica Solutions. By H. ONSLOW, Trinity College, Cambridge **266**

Obituary Notice of Fellow deceased:—Keith Lucas (with portrait) **xxxi—xlii**

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HARRISON & SONS, 44–47, ST. MARTIN'S LANE, LONDON, W.C. 2.
Price Four Shillings.

No. B 628.

July 1, 1918.

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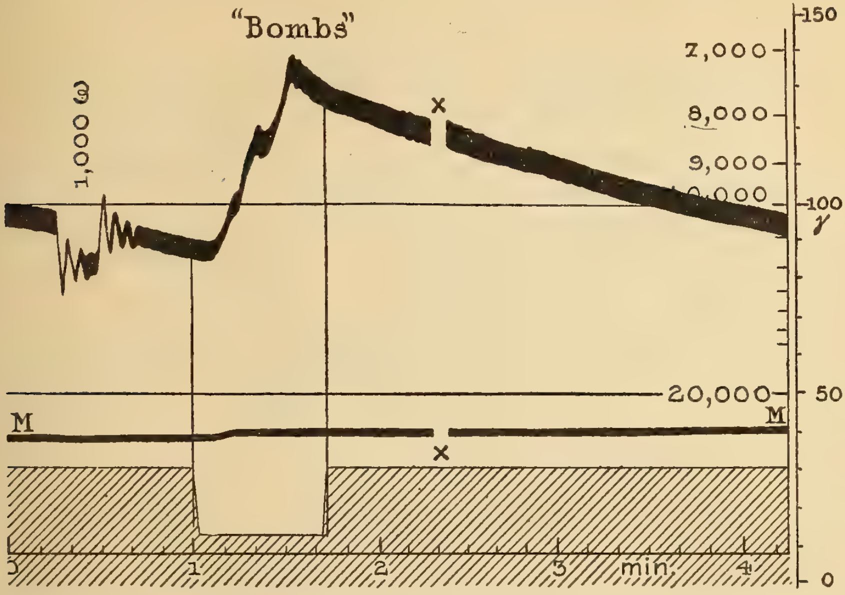

FIG. 2.—Emotive response to the suggested idea of an air-raid. The brief interruption of light marked X was made for the purpose of testing the record for parallax.

In both records the battery (two Leclanché cells), patient, and galvanometer (shunted) are in series.

In other observations I have followed the more convenient method of placing the patient in the x arm of a Wheatstone bridge and calibrating by 0·14 volt whatever departure from zero (lowered resistance of x) is produced in response to the emotive stimulus.

Different subjects react differently to different stimuli, and the same subject in different states of health and at different times of day reacts differently to identical stimulation.

On the Quantitative Differences in the Water-Conductivity of the Wood in Trees and Shrubs. Part I.—*The Evergreens.*

By J. Bretland Farmer, F.R.S., Professor of Botany in the Imperial College of Science and Technology.

(Received January 23, 1918.)

The present communication aims at presenting, in a necessarily curtailed and somewhat preliminary form, the results of an enquiry into the comparative efficiency of the wood as a water-conducting tissue in about sixty species of plants. These consisted for the most part of trees and shrubs, but a few herbaceous forms were studied as well. The investigation was undertaken in the first instance in order to find out whether the efficiency (regarded from the standpoint of its water-conductivity) of the wood could be usefully expressed for a given species in a quantitative form, and if so, what kind of deviation from the average or mean was to be expected, and to what extent separate species might differ among themselves in this respect; secondly, whether the mean conductivity can be correlated with any obvious character such as deciduous or evergreen habit; thirdly, to ascertain if possible whether definite changes of external conditions can evoke corresponding change in the water-conductivity.

It is a remarkable circumstance that, although the absorption of water by the roots and its elimination during transpiration from the leaves and other green surfaces are processes which have served as the starting-points of a vast number of investigations, the behaviour of the wood as the intervening conducting channel has been almost entirely neglected. It is true that the path actually followed by the water has long been known, and that attempts to discover an adequate physical explanation of the ascent had been repeatedly made for many years before the fine researches of Dixon and Joly showed clearly where the solution of this problem was to be sought. But the limitation which the structure of the wood may impose on the volume of water transmitted appears to have attracted no attention at all. And yet the life and habits of a plant are so closely linked up with the problem of water supply that every factor that can influence the complex adjustment of supply of and demand for water deserves serious consideration. It cannot be devoid of interest for those concerned in arboriculture and forestry, for not only may it determine whether a particular species can flourish under given natural conditions, but it also concerns the inter-relations between those tissues in the wood which materially determine the nature and commercial value of timber.

The method adopted for securing the data required for the present investigation consists essentially in measuring the amount of water passing in a given time and at standard pressure through a definite length of twig or branch, the cross-sectional area of whose wood is carefully ascertained. From the data thus obtained, it is easy to state the result in terms of the amount of water that would pass under precisely similar conditions through a stem, the cross-sectional area of the wood of which measured 1 sq. cm. in cross-section; in other words, starting from a ratio, "absolute volume" ÷ observed area of wood, to state the results in such a form that "area" shall equal 1 sq. cm. The actual procedure adopted was as follows:—

A branch of a tree or shrub is immersed in and severed under water, in order to obviate the entrance of air into the vessels. Twigs from ½ to 1 cm. in diameter are cut off, also under water, and are divided into lengths of exactly 15 cm. In the case of rod-like branches, *e.g.*, of sapling trees or coppice-shoots, it is possible to get from three to five or more lengths, and these are kept together and numbered from base to apex for purposes of subsequent identification. Any lesions consequent on trimming off side branches are well luted up with sealing-wax of low melting point, this proving the best of all the substances tried. The twigs are then put into a vessel of boiled water and subjected to vacuum treatment, in order to free the wood as far as possible of any air it might contain. This precaution has served to eliminate most of the discrepancies which appeared in the earlier stages of the investigation. The twigs are then fixed (wiring them if necessary) by their lower or basal ends into the pressure tubing at the ends of the vertical tubes below J, J_2, J_3, J_4, in the apparatus shown in fig. 1, and the amount of water, delivered at a pressure of 30 cm. of mercury, which is transmitted in exactly 15 minutes, can easily be collected and measured in the graduated glasses below. Three or four successive measurements were made for each twig in order to test the uniformity of flow, which thus lasted for about an hour or more. Only those results which showed no, or very small, deviations were accepted, but the number of those discarded as untrustworthy becomes very trifling when precaution is taken to prevent air trouble. Stems which contain resin or mucilage, however, are apt to be unsatisfactory, owing to the blocking of the ends of the water-conducting tracts. After the stems are removed and the estimation of the transmitted water is finished, the cross-sectional area of the wood is measured. The twig is bisected transversely, and a section from the surface so exposed is prepared for the microscope. The limits of the wood are accurately traced on paper by means of a camera lucida at an exact magnification of 10 diameters. By means of a planimeter the area of the wood thus obtained can easily be determined. Inasmuch as the instrument used by me measures areas in inches a reduction to centimetres has to be made, and the result divided by 100 gives the real area in square centimetres.

It may be objected that a section taken from the middle of the stem will not give absolutely accurate data for estimating the flow per square centimetre through the twig. But, having regard to the natural taper of the stem and the fluctuating area of the wood, it is probably as near an approximation as it is possible to reach. Furthermore, as all the specimens are treated in the same way, the error, in so far as it affects the value of comparative results, will in any case be very small, and well within the limit of unavoidable experimental error. From the data obtained as above, the volume transmitted by a stem 15 cm. in length per 1 sq. cm. of wood (in cross-section) is calculated, and this ratio constitutes what is hereafter termed the "specific volume," and it expresses quantitatively the "specific conductivity" of the wood of any given twig. Comparison can be made with other branches, whether of the same or another species, thus enabling a very precise estimate to be made of the range of variation occurring in a particular species, and this again serves as the basis for comparing the behaviour of different species with one another.

The apparatus employed in this research is shown in the accompanying illustration (fig. 1), for which I am indebted to Miss Reeks, Technical Artist. It consists, essentially, of a tube which conveys the water, under any desired pressure, to the twigs figured as dipping into the four measuring glasses. A special arrangement also renders it possible to suck water up through the twigs from the measuring glasses, and to determine the amount so transmitted by means of the burette marked P in the figure.

The water is supplied from the main supply by means of the tap A; it passes through a glass wool filter D_1 to a T-piece, and thence in the direction of the arrow through the tap E, and a second glass wool filter D_2 to the three-way tap F, which connects with the manometer. The water rises in the tube leading to the latter, but is prevented from passing into the manometer head by the two pairs of bulbs G_1 and G_2. The three-way tap F further carries the water through H to the four twigs through the three-way taps J_1, J_2, J_3, J_4. Any or all of these twigs can, of course, be disconnected at pleasure from the water in the horizontal tube leading to the three-way tap K, which ordinarily serves as a cut-off, and thus all the water is delivered through the twigs, and the amount from any or all of them is severally collected in the measuring glasses. Any air bubbles that may occur in the system are easily washed out by opening the taps K and O, and drawing the water through the apparatus by means of the filter pump B attached to the middle of the three main taps. For this purpose the bottle of water N is specially useful, as a means of completely washing out the whole of the horizontal tube.

The pressure of water from the main is kept nearly constant by the device of connecting the limb of the T-piece beyond D_1 with a filter pump attached to the main tap A. The negative pressure thus produced is roughly adjusted to carry off a part of the water that enters from A, and a screw pinch-cock C serves as a very delicate fine adjustment. The desired pressure within the apparatus, registered by the manometer shown in the figure as graduated in centimetres (but actually each centimetre is subdivided into millimetres), is thus easily obtained, while the sort of differential pressure in the T-piece secures steadiness, since any sudden increase in A is largely met by an increased outflow in A, and thus is prevented from effecting sudden change in the body of the apparatus. But, although the manometer level may remain constant for a considerable period, any unusual demand on the main supply naturally becomes reflected in a change in pressure, but this can instantly be remedied by means of the pinch-cock C. It need hardly be added that the height of the water from the bulb G_1 to the twigs must be taken into account in determining the height of the mercury required to indicate the particular pressure desired.

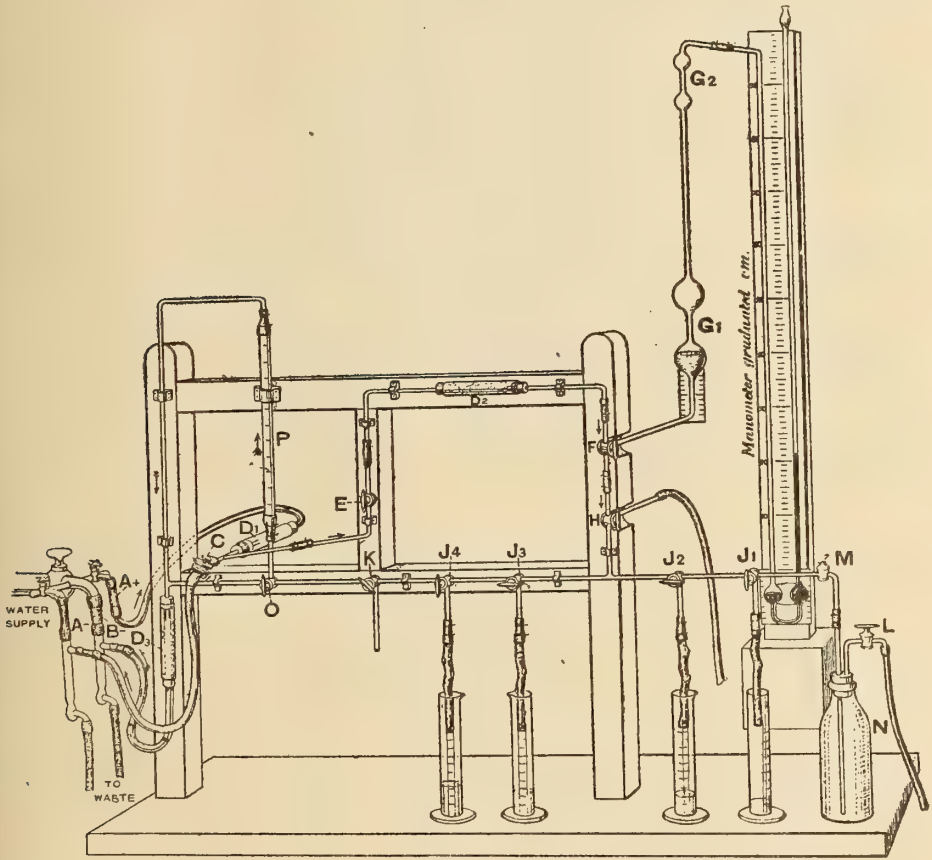

Fig. 1.

When the apparatus is used to measure the amount of water sucked up through a twig from a glass, under given conditions of negative pressure, the procedure is somewhat different. For this purpose all the twigs are first cut out of the water circuit by the three-way taps. The water supply is then cut off at the tap F, which at the same time places the manometer in communication solely with the part of the tube below it.

The negative pressure is obtained by connecting the horizontal tube with the filter pump B through the taps O and K. Any air bubbles that may be formed are easily washed out by temporarily admitting water either through H or M. Practice enables one to judge how much water is needed in the system to correspond with any desired pressure, and, having once obtained this amount, the tap O is turned to direct part of the water into the burette P; finally, any additional water required to bring the water-level therein to the zero mark is drawn in through K. Only one twig can, of course, be connected up during a single experiment, and care must be taken that the height of the water column on the scale G is always brought to the same level when readings of the burette are to be taken.

In practice, the results obtained under negative pressure merely confirm those more easily and economically (since four twigs can be used simultaneously) reached under positive pressure. This fact indicates that, as might be expected, the water is transmitted with equal facility from base to apex and in the reverse direction, having regard to differences of thickness of the two ends. It serves as a useful check on results where there is reason to suspect air bubbles as the cause of unexpected irregularities in consecutive readings, for the alternate application of positive and negative pressure may be used to dislodge them without otherwise interfering with the experiment. At other times also it affords a useful means of testing a reading of unanticipated value.

The subjoined Table indicates how the records were made, and the degree of uniformity to be expected. It may be noted that the doubled pressure (60 cm. mercury) is only applied for 7½ minutes, that being equivalent to 30 cm. for 15 minutes. The readings are taken to the nearest tenth of a cubic centimetre in well-made measuring glasses, graduated in cubic centimetres and tenths of a cubic centimetre. The stem in question happens to be that of a deciduous tree, but it has an advantage in this respect over that of an evergreen, in that the larger actual values given afford a better illustration of the method as a whole.

Table I.—Apple, Cox's Orange Pippin, September 27, 1917. Length of Stems, 15 cm.

Time.	Stem No. 1.	Stem No. 2.	Stem No. 3.	Stem No. 4.	Pressure.
4.3 to 4.18 P.M.	11·4	10·6	7·6	4·8	+30 cm. mercury
4.19½ to 4.27 P.M.	11·2	10·6	7·2	4·6	+60 cm. „
4.28 to 4.43 P.M.	11·6	10·6	7·4	4·8	+30 cm. „
Average absolute volume	11·4	10·6	7·4	4·8	
Area of wood in middle zone of twig (Planimeter reading)	2·68	2·91	2·06	1·47	

The area of the wood is given in inches, as measured by the planimeter on a drawing to scale (×10) from a section. It is reduced to cm. at magn. (×1) in calculating the specific volume.

Perhaps the most clearly defined result which has emerged from this investigation is that the evergreens, as a class, possess wood of markedly lower efficiency than that of the broad-leaved deciduous trees. In other words the specific conductivity is lower. Furthermore the absolute range of variation in an individual species is far narrower than in the deciduous forms. The Holly affords a good example of this character. The specimens used for the experiments were cut from trees and bushes of the most diverse habitat, some growing in wet soil near a brook, others in woodland shade, others again were hedgerow trees on well-drained sandy soil, fully exposed to wind and sun. No constant difference was observed between shoots bearing prickly leaves, and those with laurel-like foliage. The subjoined Table will illustrate the points just mentioned, and it also shows that the uniformity of the wood is independent of its cross-sectional area and the age of the twig. Of course, in this, as in all the other examples, only the sap wood is under consideration. No branches with functionless heart wood were used. Naturally also, properly ripened wood must be taken. Young or immature wood always gives a relatively low reading and of quite uncertain value.

Table II.—Twenty-eight Twigs of Holly from Different Trees Growing in Diverse Situations.

Age in years.	Area of wood in inches (× 10).	Absolute vol. in c.c. per $\frac{1}{4}$ hour.	Specific vol. in c.c. per $\frac{1}{4}$ hour.
1	1·60	1·1	10·8
1	1·45	1·0	10·5
1	1·19	0·6	7·8
1	1·36	0·7	8·0
2	2·74	1·6	9·0
2	3·48	1·7	7·7
2	6·19	3·0	7·5
2	4·84	2·6	7·0
3	6·36	5·0	12·2
2	1·93	1·4	11·3
1	2·56	1·5	8·9
3	3·74	1·9	7·8
1	2·49	1·3	8·1
1	1·59	1·2	9·8
1	1·77	0·9	7·9
1	1·24	0·7	8·6
2	4·75	3·3	8·6
2	3·30	1·8	7·4
2	3·28	2·0	9·4
2	2·60	1·6	7·9
2	1·98	1·2	9·4
2	2·47	1·4	8·6
2	2·49	1·2	7·9
1	1·52	0·8	6·8
2	4·73	2·3	7·6
2	4·23	2·7	10·0
2	4·34	2·2	7·8
2	2·72	1·9	8·6
	82·94	46·6	242·4
	(= 5·35 sq. cm., actual size)	$\frac{46·6}{5·35} = 8·7$	Average = 8·7

The value for the wood of the Yew (*Taxus*) was also low, but slightly higher than that of Holly, with specific conductivity of 12 ± 2 in the rather small number (6) of twigs examined, but the ages of these ranged from three to eight years, and I think the result may be taken as fairly accurate. *Podocarpus Milanjiana* grown in a plant-house in the Physic Garden at Chelsea was also examined. The plant was a well-grown one, and the branches were each divided into a basal and apical half. The average of the specific value of each pair thus obtained was also strikingly uniform, and it illustrates the desirability, in work of this kind, of getting fair averages to use as a basis of comparison. There are often considerable fluctuations in the conductibility of different regions of lateral branches, but when the average of the whole length is taken a figure is reached which is very near the mean for the particular species. This is much more obvious in deciduous than in evergreen trees, as in the former the fluctuation is considerably greater. It will be seen that in eight branches of *Podocarpus* the mean is 9·3 with an extreme variation of only 1·1 if the weak fourth shoot (at 7·1) be omitted. The Pine (*P. sylvestris*) and Larch (*L. europæus*) presented difficulties, owing to the presence of resin, but the figure for their specific conductivity is respectively about 14 and 16. Such uniformity as that indicated for the species hitherto mentioned is by no means universal even in the evergreens. Thus 15 stems of *Garreya elliptica*, with an average of 14·6, showed a

Table III.—*Podocarpus Milanjiana.* Each branch is divided into a basal (L) and an apical (U) half, and the results of each pair are bracketed.

Order of segment.	Age in years.	Absolute vol. in c.c. per ¼ hour.	Specific vol. in c.c. per ¼ hour.	Averages.
L	3	0·9	8·8	= 8·90
U	3	0·7	9·0	
L	5	1·4	8·9	= 9·65
U	5	1·4	10·4	
L	7	2·0	10·3	= 9·65
U	4	1·1	9·0	
L	4	0·7	7·2	= 7·10
U	4	0·5	7·0	
L	4	1·8	9·9	= 8·55
U	4	1·2	7·2	
L	4	1·2	9·2	= 10·35
U	4	1·3	11·5	
L	6	3·4	9·9	= 10·30
U	5	3·2	10·7	
L	4	1·0	8·9	= 9·70
U	4	0·9	10·5	
		Total		74·10
		Average		= 9·27

± variation of 5, whilst 50 stems of *Rhododendron ponticum* taken from different localities chiefly varied from 10 to 25, with a large proportion grouped about 18. But some were lower than 10, and a few were much higher than 25. Such variation is to be expected in a plant which is so freely branched, and with so wide a difference in the development of the branches, extending almost from abortion to extreme luxuriance.

Euonymus japonicus is also worth considering in this connection, and especially so because it will subsequently be convenient to compare it with the deciduous *E. europæus.* The branches of which the results are given in the annexed Table were taken from four sets of bushes, one lot growing in sandy soil in Surrey, the others from good fertile garden soil in Buckinghamshire, the last one growing in well-manured ground under the shade of trees. Although the plant indicates considerable responsiveness to the influence of the environment, it still is well within what might be called the evergreen limits, and in this respect contrasts with the deciduous species. Taking the mean for *E. japonicus* to be 12, it is seen that of 18 stems, 12 fall within 12 ± 3, while three are above and three below these limits.

Table IV.—*Euonymus japonicus.*

Age in years.	Absolute vol. in c.c. per ¼ hour.	Specific vol. in c.c. per ¼ hour.
1	0·6	12·5
1	0·5	7·5
1	0·5	10·2
2	1·4	9·0
2	1·0	8·9
1	0·6	9·5
1	0·4	8·6
1	1·4	13·4
2	0·7	11·6
2	0·8	11·3
1	0·8	13·4
1	0·6	10·9
1	0·3	9·8
2	5·2	19·1
3	4·2	17·0
2	3·9	17·1
3	3·1	14·9
1	1·5	13·9
Total		218·4
Average		= 12·1

The heaths are, as might be expected, rather low in the scale. The large heath (*Erica arborea*) is the highest with an average of 15 and a range of ± 5. This may possibly be connected with ease with the lesions that often occur in the wood of this plant. *Pernettya,* on the other hand, gave an average (six observations) of 5 with scarcely any deviation.

Escallonia macrantha, as might be expected from its habit, is higher up in the scale with an average of 24, and 6 of the 7 stems investigated were inside the range of ±2. The deciduous *Azalea mollis* averaged 30·5 with a range in 8 of the 9 stems examined of ±3·5.

The species of the genus *Daphne* are of somewhat special interest as regards their relations to water. They are slow-growing plants, and include both evergreen and deciduous species. Among the latter the common *D. Mezereum* shows a near approach to evergreen habit, and lacks the abundant foliage of ordinary deciduous shrubs. In short it belongs to the class of plants often termed xerophytes, because they are supposed to be adapted to a dry environment, and yet it is a matter of common experience that the plant does not really thrive under xerophytic conditions, and this is even more true of actual evergreen species such as *D. Blagayana, D. Laureola,* etc. It is true that these will live in soil that is well drained, but they are by no means tolerant of drought. *D. Laureola* is commonly a woodland plant, and it grows best by the side of water. *D. Blagayana,* a plant that is sometimes difficult to grow, often refuses to succeed when exposed to sun and drought in the drier climate of the Home Counties. It requires to have its stems layered and to be earthed up with stones and leaf mould. If this is not done the shoots are apt to die back by reason of the inadequate supply of water which reaches the terminal cluster of rather large leaves. When earthed up, however, adventitious roots spring from the stem and make good the deficient water supply. For not only is the supply more copious, but it is also more immediately at the service of the leaves by reason of the shorter distance to be traversed through the wood.

All the species I have examined possess a wood of very low conductivity. *D. Mezereum,* as might be expected, is the highest, at about 7.

The low conductivity is clearly connected with the slow growth, and, like the latter feature, is a strongly marked and inherent character of the whole group. Nevertheless, the plants can hardly be, with propriety, termed xerophytes. The rather large leaves of the evergreen species are badly fitted to resist transpiration. They are reduced in number, it is true, but those present actually transpire rather freely. It is unquestionable that the limiting effect of the wood materially influences the reduced leaf area, although questions as to root efficiency are also bound up with the matter, at any rate in terrestrial plants. Those leaves that *are* present require supplies enabling them to transpire somewhat freely. Hence it becomes intelligible why a plant apparently xerophytic may yet be restricted to localities in which it is never really subjected to drought. The wood is capable of transmitting a limited amount of water, the leaves are fitted just to utilise this amount with very little margin; in other words, though the plant requires little, it can hardly do with less. Furthermore, the roots are able at all times to supply the small amount of water needed by the relatively small, or else slowly transpiring, leaf surface, and this circumstance is doubtless the prime factor both in determining the evergreen habit, and also in limiting the situations in which the plants can flourish. The subject of the interrelation of root, stem, and leaf is a very complex one, and obviously cannot be dealt with here. It must suffice to have indicated that the stem structure may exert an influence in the whole problem which is by no means negligible.

It may be mentioned, also, that the creeping habit, combined with rooting at the nodes, so often to be seen among herbaceous plants, is also commonly connected with a wood of defective conductivity. It is not improbable that this correlation, which experience indicates very generally to exist between the creeping habit on the one hand, and with badly conducting wood on the other, is not merely a coincidence. Plants differ widely in the degree of variability in this or that character, and creeping plants would certainly repay experimental treatment from the point of view here under consideration.

Another plant of special interest in the present connection is the Butcher's Broom (*Ruscus aculeatus*). Attention must be called in the first place to the fact that it belongs to the class of monocotyledons, and the structure of its stem is unlike that of the plants hitherto considered. Owing to the circumstance that there is no clear demarcation between the wood and the cortex without and the pith within, it is not easy to get a well-defined area for measurement. The plan adopted was to include all the tissue within the cortex as wood. This doubtless (as in the case of other monocotyledons) means that the figure arrived at as representing the specific conductivity is somewhat low. But it seems the best that can be done, and having regard to the low absolute conductivity it is probable that it is not so far from the correct figure as might be supposed. At all events the monocotyledons themselves are fairly comparable *inter se* on such a basis.

The Butcher's Broom is certainly one of the most striking examples of a xerophyte, so far as habit goes, among all our native plants. And yet it avoids habitats which might be regarded as consonant with its appearance. Like the Daphnes, it requires very little water—far less, relatively speaking, than they do. But it flourishes best in woodlands, and thrives in damp ground, soon disappearing when really dry conditions set in, even though these may endure for short periods of time. There is no question of invoking the facile "explanation" that it is adapted to "physiological drought." Its associates are mainly ordinary mesophytic, often almost hygrophytic, plants, and no assumption of sour soil, or of unavailable or peaty water will satisfy the facts.

Suggestions as to the supposed influence of "sour" soil have often been advanced to account for the supposed xerophytic habit of rushes and sedges. Nearer examination tends to show that such "explanations," however attractive, are apt to be illusory. Neither rushes nor sedges are for the most part at all adapted to endure real shortage of water.* It is true that some of them use relatively little, but they are commonly extremely intolerant of any diminution beyond their actual modest requirements. It is quite possible to grow rushes in apparently dry garden soil, provided the water table is not so far down that the capillary action of the soil is not at all times able to supply the roots with the amount the plants normally utilise. The small amount of water actually rendered available is to be attributed to the defective conductive capacity of the wood, which in all such plants as Butcher's Broom, Rushes, etc., so materially hampers a free supply. The same is true of the aquatic *Scirpus lacustris*, with its green wand-like stems, and also of the Equiseta. None of these plants are able to endure even short periods of complete deprivation of water without wilting.

It appears then that the xerophytic habit of the plants just mentioned is to be correlated very directly with the inherent structure of their own wood, and not with any special feature in their external surroundings, or in imperfect water absorption.

An inspection of the figures given for *Ruscus* will readily serve to make clear the points alluded to above. Indeed so little water is transmitted per $\frac{1}{4}$ hour, that it was necessary to continue each measurement for an hour, or else to double the pressure for half-an-hour (which yields the same result) in order to collect enough water for a reliable estimate to be made.

The climbing plant *R.* (*Semele*) *androgynus* is strictly comparable with *R. aculeatus* in respect of its stem, and as might be anticipated it is found to possess a vastly superior vascular tissue, regarded from the point of view of its conductivity. Like the climbing species of *Asparagus*, which have also unquestionably sprung from xerophytic ancestors, it has largely increased its green water-transpiring surface and it is freely branched. Its specific conductivity varies within rather irregular limits, but the mean is about 40 in

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* In an interesting paper, by Dr. M. Delf, the point is made that the leaves of halophytes are not specially adapted to restrict transpiration. It would be of interest to examine the wood of some of these plants. See Delf, "The Meaning of Xerophily," 'Journ. Ecol.,' III.

Table V.—*Ruscus aculeatus.* Eight stems, of which seven are divided into a lower and upper half.

	Absolute vol. in c.c. per ¼ hour.	Specific vol. in c.c. per ¼ hour.	Average.
1. Entire	0·2	0·77	0·77
2. Lower	0·2	0·76	0·65
Upper	0·1	0·49	
3. Lower	0·3	1·15	1·06
Upper	0·2	0·96	
4. Lower	0·15	0·81	0·93
Upper	0·15	1·04	
5. Lower	0·2	0·85	0·82
Upper	0·1	0·78	
6. Lower	0·15	0·75	0·77
Upper	0·15	0·78	
7. Lower	0·2	1·10	0·88
Upper	0·1	0·74	
8. Lower	0·2	0·85	0·94
Upper	0·2	1·12	
Mean			= 0·86 (nearly)

the material at my disposal, the stems of which were about 0·4 cm. in diameter. Although very much greater than that of its shrubby relatives, its conductivity still falls far below that of the common dicotyledonous climbers. It is probable that the rather clumsy and characteristic appearance of most arborescent monocotyledons is to be associated with a defective water conductivity. The Palms, for example, commonly have leathery leaves and unbranched stems. There are, however, some exceptions amongst the palms, and it would be of great interest to know more about the efficiency of their wood.

Among dicotyledonous climbers the Ivy deserves notice, since it illustrates a feature that is very characteristic of climbers in general. The wood of its creeping youth-form has a low conductivity value, ranging round 25, but as the plant reaches the freely branching large-leaved adult form ("Tree Ivy"), the vascular tissue undergoes a change, and the conductivity increases to at least twice the value of that characteristic of the juvenile stage. But the relatively inadequate water supply is seen in the inability of the plant to succeed in exposed dry situations, and by the reduction in the size of its leaves plainly to be observed in Ivy that is freely open to sun and wind.

The common Privet (*Ligustrum vulgare*) stands high among the evergreens as regards conductivity. It often sheds its leaves in winter, and may, therefore, be regarded as approaching the deciduous class. Its principal range, as determined from an examination of 50 stems, extends from 30 to 42,

Table VI.—Twenty-three Branches of Privet.

Age in years.	Absolute vol. in c.c. per $\frac{1}{4}$ hour.	Specific vol. in c.c. per $\frac{1}{4}$ hour.
1	6·0	37·5
1	7·4	35·5
1	7·2	34·5
1	5·2	35·5
1	6·4	39·0
1	6·5	34·5
1	6·2	41·5
1	6·3	29·2
2	9·0	31·0
2	8·0	40·0
1	7·6	37·5
1	7·6	39·0
2	11·4	33·5
2	7·6	42·0
2	14·8	37·0
4	14·6	32·0
2	14·6	40·5
2	4·0	32·5
4	8·0	27·5
2	11·2	37·5
2	16·2	38·0
2	9·4	37·5
2	15·0	34·0
Total		826·7
Average		= 35·9

with a few outliers on either side, but a large proportion falling close to the mean, 37·3, with a P.E. of 3·6 for the whole lot.

The curve is a very regular one, but not more so than that furnished by

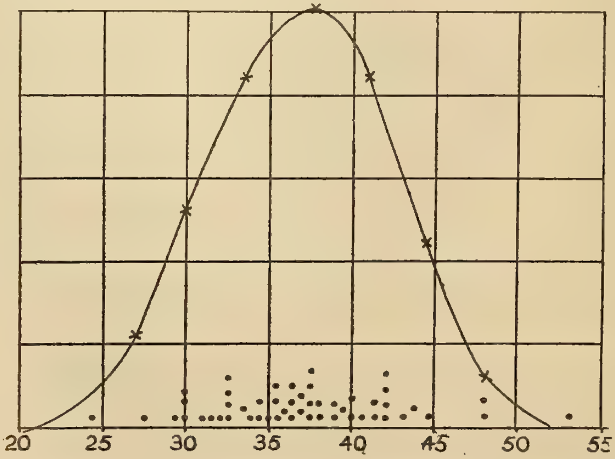

Fig. 2.—Results for Privet. Arithmetic mean = 37·3 and the P.E. = 3·6.

Summary of Observations on Evergreens, Trees and Shrubs, etc.

Name of plant.	Range within which fluctuation is probably normal.		Results falling outside range. Highest and lowest deviations are included in brackets.				Ratio of number falling within normal range to those outside it.	Totals.
	Range.	Number included.	Below normal range.		Above normal range.			
Larch	14·5±2	10	1	(9)	2	(17·6)	10 : 3	13
Scots Pine	13±2	2	—	—	—	—	2 : 0	2
Yew	12±2	4	—	—	1	(14·8)	4 : 1	5
Podocarpus Milanjiana	9±2	18	—	—	1	(13·2)	18 : 1	19
Eleagnus macrophylla	15±5	6	1	(8)	—	—	6 : 1	7
Privet	37±8	46	2	(24)	3	(53)	46 : 5	51
Daphne Laureola	3±1·5	17	1	(1)	2	(6·5)	17 : 3	20
,, *Mezereum*	6·5±1·5	13	1	(3·5)	1	(9·8)	13 : 2	15
,, *Blagayana*	7·5±1·0	2	—	—	—	—	2 : 0	2
Veronica Traversii	11±1·5	3	1	(9)	1	(15)	3 : 2	5
Euonymus japonicus	12±6	31	1	(4)	—	—	31 : 1	32
**Rhododendron ponticum*	18±10	67	9	(5)	19	(48)	67 : 26	93
Holly	9±2	35	3	(5)	—	—	35 : 3	38
Garrya elliptica	13±3	10	—	—	5	(18)	10 : 5	15
**Quercus Ilex*	32±12	14	5	(8)	3	(62)	14 : 8	22
Prunus Laurocerasus	10±2	6	2	(7)	1 (abnormal)	(18)	6 : 3	9
P. lusitanica	18·5±4	10	2	(10)	2	(26·9)	10 : 4	14
Common Broom	25±5	6	2	(13)	2	(39)	6 : 4	10
†*Azalea mollis*	30±5	8	—	—	—	—	8 : 0	8
†*Lilium Martagon*	17±7	7	—	—	—	—	7 : 0	7
Arborescent Heath	8±4	6	—	—	1	(25)	6 : 1	7
Pernettya mucronata	5±0·5	4	—	—	1	(9)	4 : 1	5
Escallonia macrantha	23±3	5	1	(18)	1	(31)	5 : 2	7
Ruscus aculeatus	1±0·3	18	—	—	—	—	18 : 0	18
Semele androgyna	Irregular							
Climbing Asparagus	Irregular							
Aucuba japonica	11±1	2	—	—	1	(17)	2 : 1	3
Olearia Haastii	14±1	2	1	(9)	—	—	2 : 1	3
Choisya ternata	11±3	24	4	(6)	3	(18)	24 : 7	31
Ivy, youth	25±5	6	—	—	—	—	6 : 0	11
,, adult	60±10	3	1	(37)	1	(78)	3 : 2	

* This is heterogeneous material, including very strong and very weak shoots.

† Not an evergreen.

observations on some of the other plants, *e.g.*, Plum "Belgian Purple." On the other hand, many species of both evergreen and deciduous plants do not admit of the water conductivity results being grouped in so satisfactory a frequency curve as that of the Privet.

The Evergreen Oak (*Quercus Ilex*) also stands high, at about 28. Its wood is far less close than that of most evergreens, and contains wide vessels resembling those of the common Oak. It shares with the latter species a considerable degree of plasticity, and in this respect it also departs from the evergreen type.

The relatively freely transpiring evergreen Portugal Laurel (*Prunus lusitanica*) has a specific conductivity of about 18·5; of 14 specimens examined 10 were within ±4 of this amount. The Common Laurel (*P. Laurocerasus*), as might have been expected, ranks much lower, and of 9 specimens 6 fell within the limits of 10 ±2.

A number of other evergreens were examined, but those here mentioned will suffice to indicate the general character of the group. The preceding Table will, however, sufficiently indicate the range of the investigation on evergreens; it will be more fully referred to in a second paper dealing with the deciduous species of trees and shrubs.

On the Quantitative Differences in the Water-Conductivity of the Wood in Trees and Shrubs. Part II.—*The Deciduous Plants.*

By J. Bretland Farmer, F.R.S., Professor of Botany in the Imperial College of Science and Technology.

(Received January 23, 1918.)

If the broad-leaved deciduous trees and shrubs be contrasted with the evergreens, it is found that they are marked by a specific conductivity which in the free-growing and foliage-bearing shoots is far greater than in the class hitherto considered.

This is well brought out by contrasting the results obtained on comparing an evergreen with a deciduous species of the same genus. The subjoined Table (I) for *Euonymus japonica* (evergreen) and *E. europœus* (deciduous) will serve to illustrate the point. It will presently become apparent, however, that there are certain exceptions to be reckoned with amongst the deciduous trees, especially in the case of young sapling trees and coppice stool-shoots (*e.g.* of ash or hazel).

Table I.

Euonymus japonica (Evergreen). *Euonymus europæus* (Deciduous).

Age in years.	Absolute vol. in c.c. per ¼ hour.	Specific vol. in c.c. per ¼ hour.	Age in years.	Absolute vol. in c.c. per ¼ hour.	Specific vol. in c.c. per ¼ hour.
1	0·6	12·5	2	10·6	29·7
1	0·5	7·5	5	6·0	31·0
1	0·5	10·2	2	16·0	38·0
2	1·4	9·0	6	12·2	46·3
2	1·0	8·9	4	16·4	48·3
1	0·6	9·5	6	8·6	51·0
1	0·4	8·6	5	13·8	45·5
1	1·4	13·4	3	7·8	47·3
2	0·7	11·6	5	11·4	45·0
2	0·8	11·3	3	5·2	39·5
1	0·8	13·4	4	21·0	53·0
1	0·6	10·9	5	4·8	26·7
1	0·3	9·8	2	9·8	37·0
2	5·2	19·1	4	8·8	40·0
3	4·2	17·0	2	9·0	29·7
2	3·9	17·1	2	14·4	37·0
3	3·1	14·9	2	10·2	33·0
1	1·5	13·7	3	8·8	45·5
Total		218·4	Total		723·5
Average		=12·1	Average		=40·2

A second feature which is brought out in the Table is that the range of actual values is larger in the deciduous trees. This is largely owing to the fact that the *proportional* range is not very dissimilar as between the two classes as a whole. But the relatively high mean in the deciduous plants makes the fluctuation more noticeable, and its absolute extent is, of course, much greater. This feature is evidently bound up with the water supplies at the disposal of the more rapidly transpiring leaves, but the inter-relations of water supply and transpiration are very complex, and will not be further discussed here, especially as they are forming the subject of separate research at the present time.

Nevertheless, the opinion may safely be hazarded that the relatively efficient character of their wood, together with its plasticity in relation to requirements, constitute, at any rate, two of the factors which have co-operated to enable the deciduous trees to assume the dominant position they enjoy in the ordinary environment within the temperate zones. Even within the tropics deciduous species are not uncommon, especially where there is an alternation of dry and rainy seasons.

The plasticity in wood-structure is borne out even within the limits of individual shoots, especially in the free-growing "extension" branches. The specific conductivity is often lower at the base than higher up, in which region the lateral leaf-bearing shoots are chiefly produced. A relatively larger proportion of the wood at the base is devoted to mechanical construction, and less to conductive purposes. It may easily happen, and, indeed, commonly does, that the absolute volume of transmitted water is greatest at the base, owing to its greater cross-sectional area. Hence, in endeavouring to get an idea of the specific conductivity of the wood as a whole, and for comparative purposes, it is best to test consecutive lengths of such branches, and then to average the lot for each shoot. The averages thus obtained coincide with one another more closely than was anticipated, and the resultant figures agree nearly with those which best express the character for a given species. In the earlier stages of the work, before this had been recognised, the figures, though obviously grouping themselves more or less round a mean, appeared to be broken up into puzzling sub-groups. This was largely due to the chance positions in a branch from which the specimens to be tested had been selected. This plasticity of the wood is a matter of great importance, which the forester cannot too clearly recognise, for, by understanding the limits of variability and the conditions that affect them, he is obviously in a better position to produce the largest yield of the most desired kind of timber. It becomes plain that, while the deciduous trees are far more plastic in respect of the quantitative distribution of the tissues composing their wood than are the evergreens, they also differ a good deal amongst each other in respect of this plasticity.

After examining the stems of Birch, Hazel, Ash, and Sycamore in sufficiently large numbers (from 100 to 300 for a single species), and plotting the results as curves, it became evident that several independent factors were concerned in producing the irregularities observed. The water conductivity was found, generally speaking, to be lower in young plants and stool-shoots, and to be often very much higher, and also far more variable (*i.e.* the wood is much more plastic), in rapidly-growing older trees. In the shoots formed on adult trees, however, the value again sank, and a closer approximation to a particular mean was obtained, in other words, individual variation became more restricted. The leading shoots, in spite of their wider average diameter, were commonly of lower specific conductivity than the laterals. This is very marked by the case when, as in the Ash, the leader commonly becomes more or less abortive, its function being assumed by one or more of the lateral branches below it. But, in any event, the laterals usually produce the bulk of the leaves, either directly or by further branching, and it is these leafy branches which always display the highest specific conductivity, at any rate, in young trees.

The methods employed in dealing with the experimental data acquired during this investigation were mainly statistical. This appeared, in the present lack of accurate knowledge on the subject, to be the most promising way to attack the subject, and to offer the best chance of clearly formulating further problems. But the conclusions drawn from the data themselves must be cautiously and critically drawn. The nature of the material, especially in the case of deciduous trees, often shows rather wide fluctuations. When there is reason to believe that these fluctuations arise owing to the lack of real homogeneity in the material, it is obvious that nothing is gained by determining the arithmetic mean and the probable error. The curves drawn from a large series of observations sufficiently indicate the interaction of different factors, and increase of the observations does not lead to a smoothing of a single curve for the total results. A frequency-grouping of values at different intervals is what is obtained, and in this way one arrives at a clearer recognition of the existence of the several influences which affect the general result.

The observations made of the Birch afford an illustration of the foregoing remarks. They show that a smooth curve cannot be obtained, nor is it likely that a very large increase in the experiments would bring this about. Even if it did the results would be misleading, because in collecting data from an indiscriminately selected lot of Birch twigs one is dealing with really heterogeneous material. The leading shoots are worse water-conductors (per unit area of wood) than the laterals, and the presence of the two maxima leads to a flattening of the curve in the intervening values. At the same time, as the subjoined Tables show, a few unusually high or low figures may seriously disturb the position of the arithmetic mean of the result if taken from such a relatively small number of specimens as 28 sets of laterals and terminals here presented.

The shoots were obtained from a lot of trees all about five to six years old, and as much care as possible was taken to secure as fairly average a set as possible both of terminals and laterals. The results show that by taking the mean of each series, it would be concluded that there was but little difference (at most only 2·8) between them—too small to be regarded as significant. But the numerical average for the terminals is really too high, and this is due to a small detached group standing at about 67. The *density* average for the terminals lies about 40, whilst that of the laterals is at about 52. The Tables give the areas as well as the absolute specific volumes, and the close agreement between the average specific volume, as recorded at the bottom of the last column, with that obtained from the sum of the areas and absolute volumes at the foot of the third column, affords a ready check on the calculations as a whole.

Table II.—Twenty-seven Birch Twigs. Leading (or Terminal) Shoots.

Age in years.	Area in inches (× 10).	Absolute vol. of water passed in $\frac{1}{4}$ hour.	Specific vol. of water per $\frac{1}{4}$ hour.
1	2·46	7·6	48·0
1	2·26	7·2	49·0
1	2·30	7·2	48·5
1	1·95	8·4	67·0
1	3·18	13·0	67·0
1	2·25	5·4	37·0
1	3·33	11·2	53·5
1	3·00	12·3	65·0
1	2·60	7·7	45·5
1	2·03	5·5	42·5
1	3·98	8·2	32·0
2	2·65	7·0	41·0
1	2·16	6·0	43·0
1	1·34	3·0	34·7
1	2·46	6·5	41·0
1	2·09	4·4	32·5
1	1·75	4·6	40·7
1	2·46	6·2	39·0
1	2·29	6·6	44·5
1	1·88	6·1	50·0
1	1·96	6·0	55·0
1	2·58	8·4	50·0
1	2·36	8·0	53·0
1	1·81	4·2	36·0
1	1·98	8·6	67·0
1	2·33	5·4	36·0
2	2·32	5·8	39·0
	63·76 sq. in. (at × 10) =4·12 sq. cm. (actual size)	190·5 $\frac{190\cdot5}{4\cdot12} = 46\cdot2\ldots$	1257·9 Average = 46·6...

With the results obtained from these young trees it is useful to compare those taken from branches cut from a tree of 14 years of age, and about 22 feet in height, felled in July, 1917.

The mean value of the position of greatest density of the numerical results both fall somewhat below that of the young laterals, but above that for the young terminal shoots.

The main average range for the Birch extends from about 38 to 58, with very distinct maximal densities round 42 and 52. This result was arrived at as the result of investigating 164 stems chosen at random. The annexed figure, in which these are shown plotted on squared paper, shows that it is hopeless to expect to get any single significant average. It will be noted that there is a small but fairly well-defined group about 63. These exceptionally high numbers may occur in twigs of any age and any diameter. They are more frequent in the Birch than in most other species examined by me, but they occur occasionally in most deciduous trees.

Table III.—Twenty-seven Birch Twigs, Lateral Branches.

Age in years.	Area in inches (× 10).	Absolute vol. per ¼ hour.	Specific vol. per ¼ hour.
2	2·34	8·2	54·0
1	1·90	6·4	52·3
1	2·30	5·0	33·5
1	2·10	7·2	53·0
2	2·95	10·0	52·5
1	2·17	6·9	49·0
2	2·44	9·2	58·0
2	2·46	8·2	52·0
1	2·53	8·4	51·5
2	2·90	7·0	37·5
1	2·78	7·2	40·0
2	2·60	7·0	47·7
1	1·64	5·8	55·0
2	1·76	5·9	51·8
1	1·30	3·4	40·5
1	1·90	7·1	58·0
1	1·80	5·2	55·0
2	1·87	5·0	41·5
2	1·60	5·0	48·5
2	2·54	8·8	53·5
2	1·40	3·6	40·0
2	2·22	5·7	39·8
2	2·06	3·6	28·0
2	3·42	12·8	58·0
2	2·57	12·0	73·0
2	1·50	4·0	41·3
2	1·30	4·8	57·0
	58·35 ins. (at × 10) = 3·76 sq. cm. actual size	184·4 $\frac{184\cdot 4}{3\cdot 76} = 49^{-}\ldots$	1311·9 average = 48·6

As another instance of variation, it will be convenient next to consider the behaviour of young sapling trees, as compared with that of the branches and terminal twigs of an adult individual belonging to the same species.

The Sycamore furnishes a good example, and I have found the same conditions that exist in it to apply more or less entirely to all other trees I have been able to examine. If well grown young trees of from four to five years of age be cut up and tested from base to apex, it becomes apparent that the wood in the upper (younger) part of the vertically growing stem is a better conductor of water per unit area than that nearer the base. But although the specific conductivity shows relatively little change, there is a great falling off in the amount of water absolutely transmitted, owing to the narrowing of the diameter of the stem towards the apex. This becomes intelligible when one reflects that saplings, until they begin to branch, commonly possess a relatively small leaf surface. The absolute amount of water transmissible

Table IV.—Sixteen Birch Twigs from 14-year-old tree felled July, 1917. All are Lateral Branches.

Age in years.	Area in inches (× 10).	Absolute vol. per ¼ hour.	Specific vol. per ¼ hour.
2	2·72	7·6	43·5
2	2·56	9·3	56·0
3	5·56	15·8	44·0
2	4·72	14·4	47·0
2	6·87	22·2	48·0
3	6·84	22·0	50·2
3	8·17	21·2	40·0
3	4·42	16·0	56·0
3	3·23	8·0	38·5
3	3·35	13·4	64·0
3	5·20	14·6	43·5
4	6·20	23·8	59·5
3	4·24	10·8	39·5
3	3·33	10·0	47·0
3	3·50	13·0	57·5
3	3·64	9·4	33·3
	74·55 sq. ins. (× 10) = 4·81 cm. (actual size)	230·5 $\frac{230·5}{4·81}$ = 47·9...	767·5 average = 47·9...

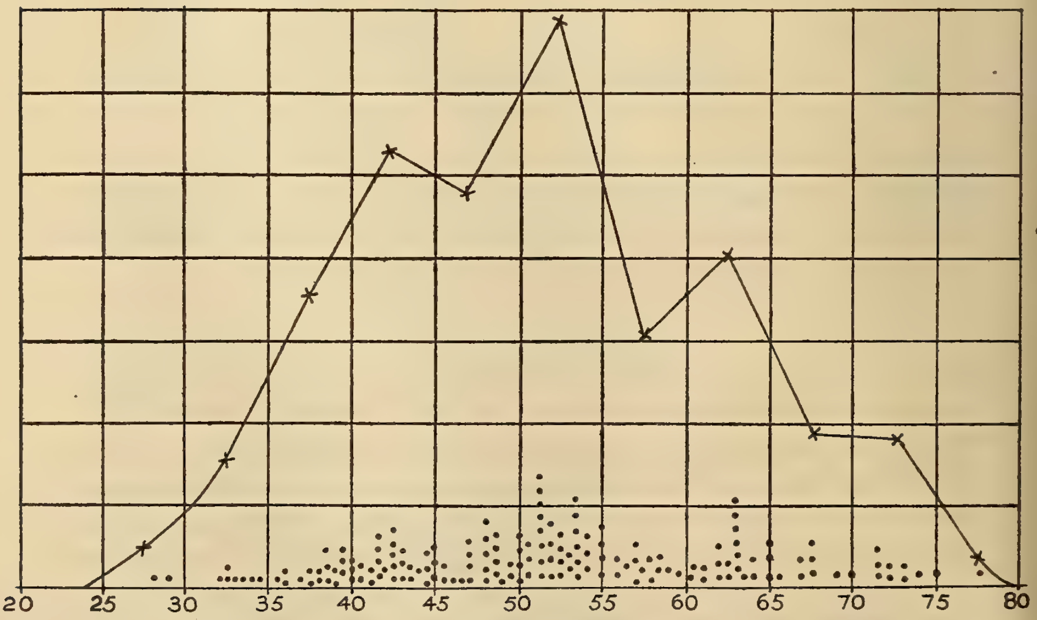

Fig. I.—Results of haphazard mixing of Birch laterals and terminals of young trees with branches of older trees.

(at whatever the pressure) represents the relative water supply available for them. The apparently unnecessarily large surplus conductivity (absolute) in the lower and leafless parts of the stem is, nevertheless, important, inasmuch as, by lowering the resistance to movement, it enables the full supply to be maintained.

Table V.—Five Sycamore Sapling Trees cut into Lengths and Tested from Base to Apex.

No. of tree.	Order of Length.	Age in years.	Absolute vol. in c.c. per ¼ hour.	Specific vol. in c.c. per ¼ hour.	Average of specific vol. per tree.
A	basal	3	18·8	29·0	36·95
	2	2	24·6	42·0	
	3	2	17·6	38·5	
	apical	1	6·8	38·3	
B	basal	2	21·0	27·0	29·22
	2	2	21·8	35·5	
	3	1	17·4	37·4	
	apical	1	6·8	17·0*	
C	basal	2	15·6	40·3	29·22
	2	2	12·4	48·0	
	3	1	4·2	33·0	
	apical	1	1·3	26·5	
D	basal	3	16·6	31·7	36·57
	2	2	16·6	32·0	
	apical	1	11·6	46·0	
E	basal	2	15·4	39·5	41·17
	2	1	11·2	42·5	
	apical	1	4·4	41·5	

The general average of the specific volumes given in the last column = 34·63; all but one of the five sets of averages fall within ±5·5 of this number.

* This apex contained immature wood, hence its exceptionally low value.

The slightly lower specific conductivity observed at the base finds an explanation in the circumstance that a relatively larger proportion of the whole wood in this region is modified to subserve mechanical requirements at the expense of its water-conducting function. The same thing also occurs, but to a very much larger extent, in the stems of young climbing plants during the period that elapses between germination and the formation of the foliage-bearing shoots. It is, of course, difficult to decide as to which is cause and which is effect in these cases, but it appears likely that the relatively ill-conducting wood which is first formed, both in climbers and in many trees, encourages the rapid upward growth of the shoot by stopping down exuberant leaf production and reducing the tendency to early lateral branching. The higher absolute conductivity noticed at the base of the young sycamore trees is largely due to the quality of the secondary wood, in which water-conducting tissue is more abundant. But the steadiness of the figures indicating specific (or relative) conductivity further emphasises the uniformity of the secondary wood in this respect, when regarded from the point of view of its composition per unit area. The Table (VI), which gives the results of comparing two lengths from the main stems of 10 young sycamores, illustrates the above points, and serves further to indicate the amount of variability that may be expected to occur in such material.

Table VI.—Ten Sycamore Sapling Trees with Sample Lengths (15 cm.) tested at the Base and near the Apex respectively.

No.	Position.	Age in years.	Absolute vol. in c.c. per $\frac{1}{4}$ hour.	Specific vol. in c.c. per $\frac{1}{4}$ hour.	Average of specific vol.
1	basal	3	4·6	20·2	24·25
	apical	1	5·2	28·3	
2	basal	3	8·4	20·3	27·40
	apical	3	9·0	34·5	
3	basal	3	10·8	34·3	36·40
	apical	2	10·2	38·5	
4	basal	3	5·0	25·8	28·65
	apical	2	4·6	32·5	
5	basal	3	4·6	20·7	28·35
	apical	2	5·0	36·0	
6	basal	4	4·6	17·8	22·55
	apical	3	5·2	28·3	
7	basal	2	6·0	25·2	25·95
	apical	1	4·8	26·7	
8	basal	3	6·8	26·2	27·10
	apical	2	5·4	28·0	
9	basal	3	10·8	34·3	36·40
	apical	2	10·2	38·5	
10	basal	3	7·2	26·6	31·05
	apical	2	7·2	35·5	

The general average of the specific volumes given in the last column = 28·81, and seven of the ten sets of averages fall within ±5·5 of this number.

The wood of the adult tree is always higher than that of the saplings, so far as my observations have extended. This is borne out by comparing the results given in Table VII with those contained in the two preceding Tables. It will be seen that the numbers for this tree vary within small limits, although the age and thickness of the twigs differed considerably. Corresponding to this difference between the basal and upper parts of sapling trees, a lowering of specific conductivity also distinguishes stool-shoots which spring from coppiced stems. This is well seen in all the instances I have investigated, *e.g.* Oak, Ash, Sycamore, Hazel, etc.

The last-named plant, the Hazel, may be taken as typical of these, and the wand-like rods that arise when the bush has been cut down very well illustrate certain characteristically recurring features in this class of stems, and in this they repeat the peculiarities of the young sapling trees as

Table VII.—Eleven Twigs from Topmost Branches of a large Sycamore Tree blown down in November, 1917. All the twigs had borne flowers.

Age of twig in years.	Absolute vol. in c.c. per ½ hour.	Specific vol. in c.c. per ½ hour.
4	12·2	41·5
1	7·2	37·9
1	10·2	34·6
1	10·0	45·6
4	11·5	47·5
3	8·2	38·5
3	12·9	43·7
2	9·6	38·6
4	11·2	47·0
3	11·4	48·0
3	11·0	47·5
Total		470·4
Average		=42·76

illustrated by the Sycamore. The wood at the base of the shoot always, or almost always, has a lower specific conductivity than that which occurs higher up. The reason lies in the relatively small amount of real vascular, and the large amount of mechanical, non-conducting, tissue. At the same time, the large diameter at the base often causes the absolute volume of transmitted water to be actually larger than that passing through the more efficient (from the standpoint of conductivity) wood higher up in the stem. As the top of the Hazel shoots is approached, both the absolute and the specific conductivity become rapidly reduced, and this is doubtless connected with many of the peculiarities of sympodial growth and branch development near the apex of such shoots, due to the dying back of the apex of the stem, and the subsequent resumption of growth by a lateral branch. The Table (VIII) shows the general type of behaviour of Hazel wands in the respects mentioned above, and it serves to emphasise the similarity that exists between sapling trees and stool-shoots—a similarity that often extends further, and embraces the arrangement and characteristic forms of youth leaves.

The Ash affords a good example of a tree in which the wood of the sapling and the coppice shoots ("ash plants") closely resemble each other in respect of their water-conductivity system, and differ greatly in this respect from the ordinary shoots which occur on the branches of the adult tree. For whereas the conductivity is great in the latter, owing to the abundance of wide and long vessels, in the former the character of evergreens is strongly recalled; even the absolute conductivity is often low in spite of the considerable thickness of the stems, but as branching supervenes, and vigorous secondary

Table VIII.—Two Long Stool-shoots of Hazel, A and B, cut into Lengths from Base to Apex.

Shoot.	Order of length.	Age in years.	Absolute vol. in c.c. per ¼ hour.	Specific vol. in c.c. per ¼ hour.
A	Basal	3	37·6	45·0
	2	3	32·0	48·0
	3	3	22·6	48·5
	4	2	12·8	38·0
	5	1	7·8	35·5
	6	1	5·0	37·5
	Apical	1	2·2	35·5
	Total			288·0
	Average			= 41·1
B	Basal	2	27·6	44·5
	2	2	20·8	47·5
	3	2	14·2	40·5
	4	1	3·8	25·5
	Apical	1	2·4	23·7
	Total			181·7
	Average			= 36·3

thickening occurs, the absolute conductivity of course undergoes a corresponding increase.

But the absolute as well as the specific conductivity of the wood falls off very rapidly as the apical region both of the leader and the lateral branch is approached. It is, I think, a fair inference that this falling off in conducting efficiency is responsible for the very characteristic habit of growth of young Ash trees and coppice-shoots. The apex often dies back for a considerable distance each year, and the elongation of the stem is assured by one or more of the stronger lateral shoots. More or less equally forked tops are not uncommon during the late spring and early summer, but one of the branches of the fork usually obtains the lead, and it is always found that its success is correlated with superior specific conductivity. A large number of examples have been employed to test these peculiarities in the Ash, as regards water-conductivity, and no exception has been encountered. Probably the best way of rendering the position clear will be found in presenting, in semi-diagrammatic form, the results obtained in a typical instance. The diagram illustrates the mode of branching, as shown in a vigorous young Ash tree growing in a hedgerow, and cut off in the early spring of 1917. The figures enclosed in brackets represent the absolute amount of water transmitted through 15-cm. lengths of the stems at the places indicated, under the same standard conditions of pressure, etc., as have been maintained throughout the enquiry. The figures opposite, which are not so enclosed, give the specific conductivity values. It is thus seen to be almost

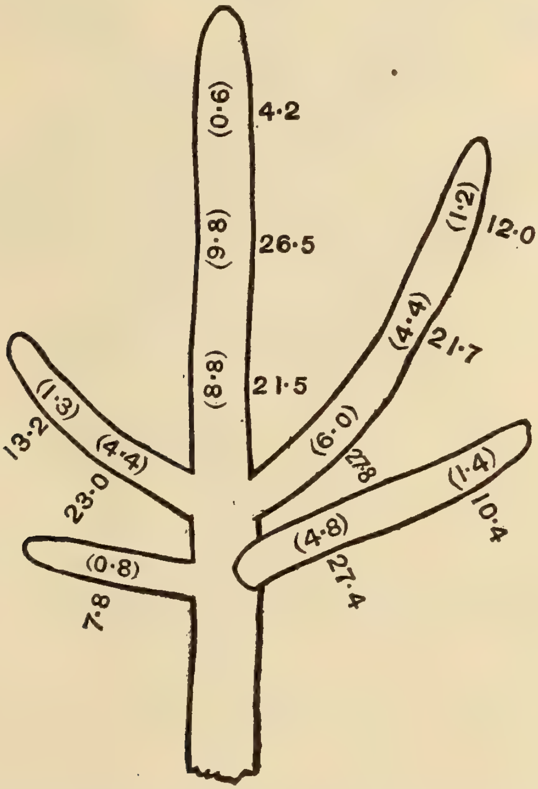

Fig. 2.—Semi-diagrammatic sketch of the top of a young Ash tree. The figures in brackets denote absolute volumes of water transmitted, those adjacent to them denote the specific volumes, at the corresponding places.

inevitable that the apex should die back, and that one or more of the stronger shoots should assume the *rôle* of leaders. The shoots further behind may grow out also, but they often fail to do so unless the stem be cut back. Of course it is not suggested that this water-conductivity factor is the only one that determines total habit; on the contrary, the final result is certainly made up of a complex of factors. But it seems clear that it does constitute an important element in determining which may and which may not become dominant shoots and branches.

In fig. 3 the top of a vigorous young Sycamore tree is represented for comparison with the Ash. It becomes at once obvious, on the basis of the explanation advanced for the dying back of the apex of the last-mentioned tree, that the Sycamore is not likely to lose its apex. And, as a matter of fact, it is very seldom that the leader in this species fails to maintain its predominance until it finally becomes merged in the formation of the crown of the maturing tree.

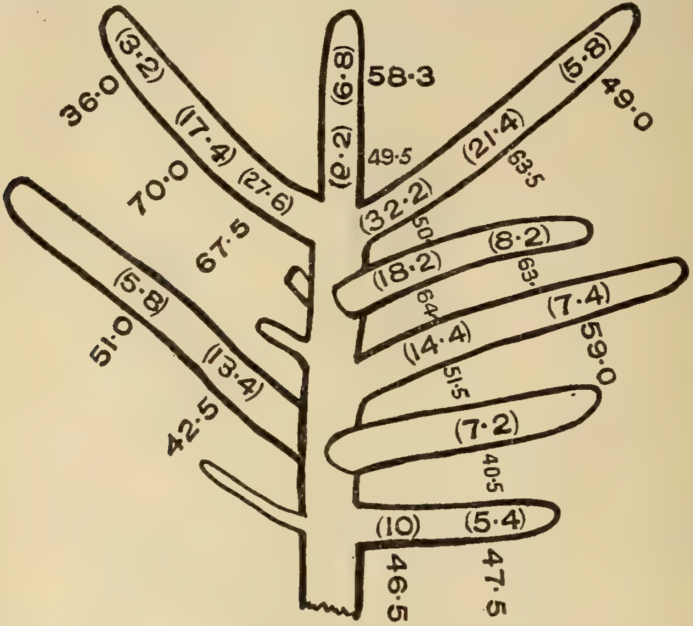

Fig. 3.—Semi-diagrammatic sketch of the top of a young Sycamore tree. The figures in brackets denote absolute volumes of water transmitted, those adjacent to them denote the specific volumes, at the corresponding places.

It is evidently unsafe to draw conclusions as to the specific conductivity of the branches of the adult tree from the behaviour of saplings or coppice-shoots of the same species. Thus, the Oak and Ash are both low in respect of the latter, but quite irregular, with very large values (from 70 to 120 or more) for the adult shoots. This great range of conductivity is by no means characteristic of all, or perhaps even of most, of the deciduous trees. Beech, Hornbeam, Elm, Sycamore, to mention only a few, are fairly regular; most Willows, on the other hand, can hardly be represented by any average number owing to their extensive fluctuation. It is just those species with wide fluctuation, however, that are likely to prove most amenable to intelligent treatment in respect of timber production, especially when it is desired to encourage the development of qualities the structural background of which is responsive to the influence of a particular environment.

A somewhat extensive series of experiments has been made on fruit trees with the view of finding out what influence manuring and pruning might exert on the character of the wood. The results as a whole are still somewhat obscure, but it is clear that the effect of manuring is greatly to increase the amount of water-conducting tissue. This may account for the exuberant vegetative development and loss of fruitfulness which often occurs as the result of too liberally manuring fruit trees (plums, apples, pears).

The observations on the results of root-pruning were not very conclusive, though they all pointed in the direction of a lowering of the specific conductivity as a result. The actual amount of wood produced on two adjacent rows of trees respectively root-pruned (in 1916–17) and not so treated, was very striking. There was a large falling off in the absolute conductivity of the total shoots for the current year, and their feeble development was clearly a consequence of the pruning. But the *quality* of the wood in the pruned trees, as measured by its specific conductivity, was only slightly less efficient as a water-conductor than that of the unpruned set of pears and apples which were used as controls in the experiments.

During the course of the investigation it was observed that a seasonal difference in the water-contents of the wood is clearly apparent in most deciduous trees. For whereas twigs and branches cut off during the summer always float in water, those cut during the autumn and winter as regularly sink at once. This means that the air which is present in many of the water-conducting elements of the wood is got rid of soon after the fall of the leaf. In the summer, while transpiration is active and the water in the tree is under low pressure, bubbles of air are formed. As was shown by Dixon, these may be re-absorbed into solution under pressure. This is doubtless accomplished by means of root-pressure in early autumn while the soil is still warm enough to enable the roots to pump up water into the trunk and branches of the tree at a time when no corresponding amount is being lost through the leaves. In this way the lacunæ (localised in individual tracheids and vessels) in the water column within the tree are again filled up with water, and the wood is already preparing to meet the requirements of the unfolding leaves in the following spring. A few trees, however, possess branches which always float, but they are those in which, as in Willows, the pith and cortex contain considerable air spaces. Of course, it is not to be expected that branches old enough to have heart wood will sink in this way, inasmuch as the heart wood has ceased to conduct water and always contains some air. But in every example I have been able to examine personally I have found the specific gravity of lengths cut from trunks containing heart wood to be very much greater in winter than when cut from trees felled in summer. This difference is largely, but not perhaps entirely, due to relative water-content in the sapwood, and it becomes a question whether, when the need for seasoned timber is urgent, it may not pay to fell during the summer in spite of the difficulties and disadvantages of so doing. Of course it is the deciduous trees that most clearly exhibit this kind of difference between the wood in summer and winter. The evergreens are transpiring all the time. and show to a far less extent the striking differences which are here referred to. I have endeavoured to obtain information on this subject from practical foresters and others * interested in woodland industry, but very little appears to be known, at any rate in this country, on the subject. Nevertheless, it would be easy to plan a few test experiments which would settle the matter, and they would also indicate the extent to which different species might lend themselves with advantage to summer felling, or how far the same drying out effect might be obtained by ringing the sapwood during the summer, as is sometimes done.

In such trees as Birches, Plums, and probably in others as well, it is possible to follow the autumnal filling up of the wood by the water. The lower branches are found to be filled first, and they sink when cut off and thrown on to water whilst those higher up still float. In a well-grown Plum-tree of about 12 years of age more than a week was required from the date at which the lower branches were first observed to sink, before the topmost twigs also ceased to float. The topmost twigs of a large Sycamore which was blown down early in November, 1917, still barely floated, while those formed lower down sank at once.

Naturally, as the soil becomes colder, the root action falls off, and it is not until the following spring that renewed activity supervenes, often to such an extent as to produce an abundant flow of sap from the stumps of trees felled at that season. It is perhaps to this latter circumstance that the widely spread idea of the wood being comparatively free from water in winter, and that the filling up of the water-conducting tissues is a concomitant of spring, is to be attributed.

In Central Europe, where the winter is more severe than in this country, R. Hartig† found the Birch to contain the lowest proportion of water at the end of September. From that time a slight rise was observed, which lasted till the middle of February, when a rapid increase occurs, and the curve indicating water-content rose sharply until the latter end of March,

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* In this connection I desire to record my thanks to Mr. Duchesne for his information given from the point of view of a practical forester, and especially to Prof. Augustine Henry, who has most kindly placed me in possession of the chief statements in the literature, the extremely conflicting character of which indicates how little actual knowledge on the subject we at present possess.

† 'Unters. a. d. forstbotan. Inst. z. München,' II.

when it began to fall again. Doubtless the difference between Hartig's observations and my own in this respect finds its explanation in the relative severity of the German winter, which checks root absorption during that period in the situations in which Birch is prevalent. This view seems to be borne out by Hartig's statements concerning the Beech. He found that the period of maximum water-content in this tree coincides with the month of December, and not with March as in the Birch. The Beech grows in less exposed situations, and its close canopy likewise serves to mitigate the effects of freezing winds on the soil. It is also of interest to note that the Scots Pine in the Central European forests behaves like the Beech, although, as might have been expected, the seasonal fluctuations in the water-content are less marked than in the deciduous tree. It is evident, however, that it is unjustifiable to assume that the results obtained under the influence of a continental climate (*e.g.*, of Central Europe, or many parts of North America) must necessarily be identical with those yielded under such widely different climatic conditions as prevail over the greater part of the British Isles.

In the subjoined Table a summary is given of the investigations on the greater number of deciduous species which have been studied in connection with this research. I have not included a number of results on fruit trees, because they form a special part of the general enquiry which is not yet sufficiently ripe for publication. No attempt has been made in this, or the preceding paper on the Evergreens, to deal with the range of fluctuation in accordance with any fixed statistical principles, but a rather arbitrarily limited range for each species has been adopted where experience seemed to indicate the limits of normal fluctuation should be drawn. It might be argued that it is useless to attempt to fix any limits other than those afforded by the actual figures, on such a variable structure as that concerned in water-conduction. But at least it is useful to recognise the relative density or frequency of occurrence of the values within the extreme limits of variability, and it is obvious that there is in most cases a genuine average value which can be assigned for each species. It is equally evident, however, that the proportional fluctuation is by no means identical for the different species. Sometimes a reason for such fluctuation can be assigned, as when heavily shaded (and consequently starved) shoots have been included in the averages.

It is not proposed at the present time to attempt a detailed discussion of the results presented in the foregoing pages. To do so adequately would require much space, and, moreover, there still remain many points on which further information is desirable, before attempting to review the whole subject. It may, however, not be out of place to state that there are good

Summary of Observations on Deciduous Trees and Shrubs, etc.

Name of Plant.	Range within which fluctuation is probably normal.		Results falling outside range. Highest and lowest deviations are included in brackets.				Ratio of number falling within normal range to those outside it.	Totals.
	Range.	Number included.	Below normal range.		Above normal range.			
Common Oak	75 ± 15	12	11	(19)	10	(240)	12 : 21	33
Beech	65 ± 10	20	3	(35)	6	(91)	20 : 9	29
Birch	51 ± 13	129	16	(18)	26	(42)	129 : 42	171
Hornbeam and stools	26 ± 6	17	1	(18)	—	—	17 : 1	45
Ordinary branches	43 ± 8	22	—	—	5	(71)	22 : 5	
Ash and stools and young trees	14 ± 10	100	7	(1)	7	(30)	100 : 14	114
Older trees	The range too wide to admit of conclusions being drawn.							
Hazel and stool shoots	31 ± 9	81	5	(16)	12	(50)	81 : 17	122
Ordinary branches	60 ± 10	26	7	(40)	1	(75)	26 : 8	
Mountain Ash and stool shoots	30 ± 10	7	—	—	—	—	7 : 0	25
Ordinary branches	63 ± 9	13	—	—	5	(79)	13 : 5	
Wych Elm stool and coppice	22 ± 7	10	2	(6)	—	—	10 : 2	12
Plum, "Belgium Purple"	45 ± 15	58	5	(20)	10	(75)	58 : 15	73
,, "Czar"	Variation very wide, correlated with excessive manuring.							
Pear, Doyenné du Comice	60 ± 20	45	9	(22)	11	(104)	45 : 20	65
Crab	70 ± 15	29	5	(32)	8	(104)	29 : 13	42
Lime	85 ± 20	22	2	(52)	4	(131)	22 : 6	28
Sycamore, young coppice shoots	25 ± 5	27	—	—	—	—	27 : 0	97
Stool shoots	42 ± 13	55	—	—	—	—	55 : 0	
Shoots from adult	65 ± 5	15	—	—	—	—	15 : 0	
Spindle tree (*Euonymus europæus*)	47 ± 8	9	1	(23)	—	—	9 : 1	10
Hawthorn	44 ± 5	8	1	(23)	1	(53)	8 : 2	10
Blackthorn	51 ± 12	10	1	(41)	1	(82)	10 : 2	12
Aspen	81 ± 11	10	—	—	2	(103)	12 : 2	12
Goat Willow	65 ± 15	22	3	(28)	9	(113)	22 : 12	34
Osier	95 ± 20	59	10	(43)	8	(157)	59 : 18	77
Apple, Cox	45 ± 15	54	9	(18)	6	(73)	54 : 15	69
,, Lane's Prince Albert	40 ± 10	21	—	—	2	(54)	21 : 2	23
Maple (*Acer campestre*)	45 ± 15	25	2	(19)	—	—	25 : 2	27
Laburnum	42 ± 10	18	3	(25)	3	(80)	18 : 6	24
Philadelphus grandiflorus, shoot from roots	20 ± 5	10	3	(9)	—	—	10 : 3	13

grounds for believing that one of the principal sources of the differences between the evergreen and deciduous trees, with their very different transpiration values, depends largely upon the smaller bore, and especially on the shorter length, of the vessels in the wood of the former group. The short length of vessels obviously involves more resistance to the passage of water. The Holly may be cited as an example. It was not possible to force clean mercury* through more than 2·5 cm. of one well-grown specimen, even at a pressure of 90 cm. of mercury continuously applied for 18 hours. On passing water in which Indian ink was suspended through another stem, the Indian ink only emerged through three vessels in a length of 6·5 cm., while at 3 cm. it had filled up 20 vessels in 18 hours at 90 cm. of mercury pressure.

Shoots of coppice Ash are also resistant, though not so effectively, the passage of mercury and Indian ink through 15 cm. length being almost entirely limited to the vessels situated close to the pith. But, as the ash sapling grows, of course the new wood is more vascular, and that of adult trees is very porous. Strasburger found he could force mercury at a pressure of 40 cm. through the wood of Oak branches to a length of 3–4 metres. The vessels of this tree are of rather exceptional length and width for a timber tree. It is possible that Indian ink or sepia suspended in water might give even higher values, as the resistance offered to the passage of mercury must obviously be considerable, unless it turns out that the mercury actually burst the thin cross walls in the vessels.

The principal results incorporated in this and the preceding communication may be summarised as follows:—

1. A quantitative method has been described for estimating the water conductivity of trees, shrubs, and herbaceous plants. The method depends on measuring the volume transmitted through 15 cm. (in length) of the stem (or root) delivered at a pressure of 30 cm. of mercury for a period of 15 minutes. The amount so transmitted is called the absolute volume. By ascertaining with precision the area in cross-section of the wood, it is practicable to reduce the absolute volume to a specific volume, which is the ratio of water volume transmitted through the stem under the foregoing pressure for 15 minutes, and an area of wood, the cross-sectional area of which is 1 sq. cm. This ratio, or specific volume, can be used as a basis for

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* Mercury, which has been used by Strasburger and others for injecting the vessels, is open to objections from an experimental point of view. I obtained far more reliable results with a specially fine sample of Indian ink, which my colleague and friend, Prof. H. G. Plimmer, was good enough to place at my disposal.

comparison between different stems, whether of the same or different species of plants.

2. The results obtained throw light on the habit of many "Xerophilous" plants.

3. The specific conductivity of evergreens is relatively low, with correspondingly small absolute fluctuation; that of the deciduous species (with certain special exceptions) is relatively high, with a fluctuation sometimes relatively high.

4. Some of the deciduous trees are markedly more plastic and are more easily influenced by environmental conditions than are others. Although this feature occurs in evergreens also, it is far less widespread.

5. A considerable difference exists between the normal adult wood of the tree and that of "leaders" of young trees, and especially of coppice-shoots. This difference, which is in the direction of a lowering of conductivity, occurs to an exaggerated extent in the main shoot of most climbers.

6. The wood of arborescent and frutescent monocotyledons is defective as regards water-conductivity, and this is to be regarded as a factor in determining their special habit of growth.

7. The wood especially of deciduous trees becomes filled up with water during the early autumn, owing to the activity of root pressure which persists after the functional activity of the leaves has ceased. It is suggested that this circumstance may have a practical application in shortening the time normally required for the seasoning of felled timber.

8. There are grounds for attributing the lower conductivity of evergreens, at least in great part, to the narrow and short vessels which are present in their wood.

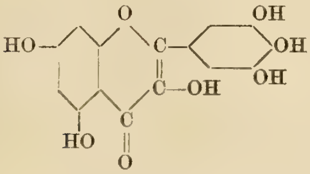
I desire to acknowledge the assistance received from the Department of Scientific and Industrial Research, which has enabled me to secure the services of an assistant, without whose help the work, owing to other demands on my time, could not have been carried on. I also wish to thank my assistant, Mr. H. Tooley, for the conscientious manner in which he has carried out much of the laborious experimental work.

The Production of Anthocyanins and Anthocyanidins.—Part III.[*]

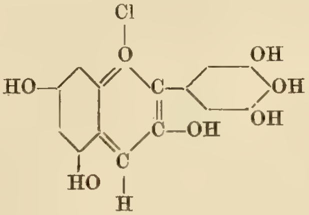
By ARTHUR ERNEST EVEREST, D.Sc., Ph.D.

(Communicated by Dr. F. W. Keeble, F.R.S. Received April 9, 1918.)

The work of Willstätter,[†] of Willstätter and Mallison,[‡] and of Willstätter and Zechmeister,[§] that has followed upon the proposal by the present author of a structural formula for the anthocyan pigments,[||] has clearly demonstrated the correctness of the formula proposed, and of the conclusions of the present author as to the relationship that exists between the anthocyan and flavonol groups, exemplified thus:—



Flavonol (Myricetin).



Anthocyan (Delphinidin).

Although it has thus been proved that anthocyanidins can be produced from flavonols, and that the flavonol glucosides pass, likewise, to anthocyanins, it does not of necessity follow that the anthocyan pigments are thus formed in plants and flowers, although this is probable, and in the present paper chemical evidence is produced which strengthens this probability.

It was with a view to an examination of some points that may prove of value in connection with the tracing of the formation of the anthocyan pigments in Nature that the author began the investigations, of which this is a preliminary announcement. In view of the fact that the work, which was begun during the summer of 1914, has had of necessity to stand aside, and must continue to do so for some time, and of the publication of further work by Willstätter and his collaborators,[¶] some of which touches upon the same ground, the author feels that the publication of the present preliminary observations will not be out of place.

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[*] Parts I and II appeared in these 'Proceedings,' B, vol. 87, p. 444; vol. 88, p. 326 (1914).

[†] 'Sitzber. K. Preuss. Akad. Wiss.,' 1914, p. 402.

[‡] *Ibid.*, p. 769.

[§] *Ibid.*, p. 886.

[||] 'Roy. Soc. Proc.,' B, vol. 87, p. 444 (1914).

[¶] Willstätter and Weil, 'Annalen,' vol. 412, p. 178 (1916).

Both Willstätter and the author have noticed that when flavonols are reduced as described by them, with formation of anthocyans, a considerable proportion of the yellow compound remains unacted upon—as the result of quantitative estimation Willstätter found, in one case, only 4 per cent. of anthocyan present after reduction—but this is, to some extent at least, due to the difficulty in regulating the reduction of the pigment, and it is quite conceivable that in Nature conditions may be more favourable to anthocyan formation than in the experiments thus far described.

It would appear desirable that evidence should be collected upon which conclusions might be drawn as to whether the anthocyan pigments are produced in plants *viâ* flavonols, or, as the result of direct synthesis, independent of the presence of flavonols.

If they are produced *viâ* flavonols, then, unless the conditions present in Nature allow the reaction causing the conversion of the flavonol into anthocyan to proceed to completion, and the reaction proceeds more rapidly than that whereby the flavonol is synthesised, and, further, that there is no reconversion from anthocyan to flavonol as the result of re-oxidation, it is to be anticipated that flowers, fruit, etc., containing an anthocyan pigment will also contain some quantity, great or small, of a flavonol, and indeed of the particular flavonol that corresponds to the anthocyan present. Thus, where the anthocyan pigment is one of the cyanidin glucosides, a corresponding quercetin glucoside would be expected, whereas if the anthocyan were a glucoside of pelargonidin, then a corresponding glucoside of kaempferol should be found, and so forth. It is, indeed, possible that if these pigments are produced *viâ* flavonols, there is a more or less regular relationship existing between the amount of flavonol and of the corresponding anthocyan present in a normal full-grown flower of any particular species when grown under normal conditions.

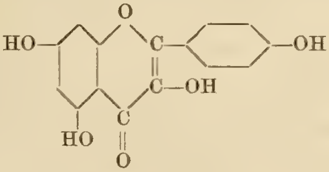
There remains a further possibility which should not be overlooked, viz., that where two flavonols are present under conditions which give rise to production of anthocyans from flavonols, one of the yellow pigments may be preferentially reduced, passing completely to an anthocyan before the second is attacked. This seems, at first sight, rather unlikely, but if such were the case, the result would be that not a flavonol corresponding to the anthocyan would be found, but one differing from it; thus the investigations proposed by the author would produce useful evidence in this case also, and, if necessary, could probably be verified by independent methods.

With respect to the presence of yellow sap-pigments, flavones or flavonols, in flowers containing anthocyans, there is considerable evidence of their co-existence already available, and many of the early workers noted that the anthocyan pigments were accompanied by yellow sap-pigments.* During the preliminary work on the cornflower pigment a yellow sap-pigment was isolated in small quantities by the author, though the product was not investigated, nor was the fact recorded in the published results,† whilst Wheldale has stated that in various varieties of Antirrhinum, anthocyans are present together with flavones, but in none of these cases have both anthocyan and flavone, or flavonol, from any one flower been isolated and identified.‡

Shortly after publication of his conclusions concerning the formation of anthocyans from yellow sap-pigments, the author began preliminary work with a view to ascertaining, in a systematic way, the identity of both anthocyan and yellow sap-pigment present in various flowers, by isolating both of these from the same batch of petals, and for this purpose, in the first instance, a series of self-coloured Violas was chosen.

Apart from the fact that the presence of yellow sap-pigments in flowers which contain anthocyans has been thus observed, there is but little evidence yet available as to the actual structure of both flavone (or flavonol) and anthocyan pigments present in any one flower.

It would appear that the case in which the published data concerning these two types of pigment most nearly approach that desired is that of *Delphinium consolida, L.* A. G. Perkin and Wilkinson,§ following up the observations of A. G. Perkin and Pilgrim,‖ isolated kaempferol



(they failed to isolate the glucoside, in which form the colour was originally present in the flowers) from the blue flowers of this plant, both when they used the flowers, as also when they made use of an extract purchased from Merck, of Darmstadt. Willstätter and Mieg,¶ who examined the anthocyan pigment present in *Delphinium consolida, L.,* using the commercial purple "Flores

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* *Cf.* Hope, 'Journ. Prakt. Chem.,' 1837 (10), p. 269; Fremy and Cloez, 'Journ. de Chim. et de Phys.' [3], vol. 25, p. 249; Filhol, 'Compt. Rend.,' vol. 39, p. 194; 'Journ. Prakt. Chem.,' vol. 63, p. 78 (1854); Martens, 'Jahrber.,' 1855, p. 657.

† Willstätter and Everest, 'Annalen,' vol. 401, p. 189 (1913).

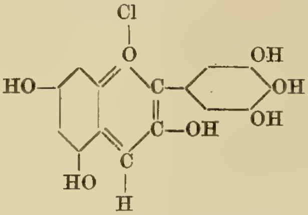
‡ *Cf.* further Willstätter and Mallison, 'Annalen,' vol. 408, p. 157 (1915).

§ 'Journ. Chem. Soc.,' 1902, p. 585.

‖ 'Journ. Chem. Soc.,' 1898, p. 267.

¶ 'Annalen,' vol. 408, p. 61 (1915).

Calcatrippæ," obtained from these flowers a complex diglucoside anthocyan, to which they gave the name delphinin, which, on hydrolysis, yielded not the anthocyan that would correspond to kaempferol (*i.e.* pelargonidin), but that corresponding to myricetin, viz., delphinidin.



In view of the fact that the colour descriptions of the flowers used do not coincide,* and that Willstätter and Mieg (*loc. cit.*) state that other varieties of Delphinium appear to contain colouring matters which differ, at least, from their glucoside delphinin, it is not certain whether the results of these researches can be safely used as evidence that in *Delphinium consolida, L.*, the anthocyan present is not that which corresponds to the flavonol derivative (yellow sap-pigment) that is also present.

Wheldale† has somewhat fully investigated the Mendelian factors in the Antirrhinum, and, in the course of her work, has isolated apigenin and luteolin from ivory and yellow varieties respectively, and extracted anthocyan pigments from various red-coloured flowers of the same plant. Here the evidence is insufficient, as Wheldale has not as yet obtained the anthocyan pigments in a crystalline condition nor identified their structure; moreover, although she assumes that certain of the red flowers contain apigenin and luteolin in addition to the anthocyan pigments, it does not appear that she has isolated the flavone derivatives actually from the red flowers, but only from ivory or yellow specimens. It is to be hoped that Wheldale will continue her work on these products, particularly in view of the fact that, if the anthocyan pigments are produced in Nature *viâ* flavone, or flavonol, derivatives, and her assumption that the red flowers contain apigenin and luteolin, as do the ivory and yellow, proves correct, then the anthocyan pigments she has under investigation should be members of a new series which are related to flavone derivatives, not, as are all the anthocyans thus far described, to flavonols.

The impossibility of assuming that, because a particular pigment is present in the flowers of one variety of a plant it will occur in those of other varieties of the same species, will be fully realised when the work of Willstätter and his collaborators on the anthocyan pigments, in particular Willstätter and Mallison,* or that of A. G. Perkin, on the various cotton flowers,† is considered.

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* The author having submitted a sample of the purple Delphinium used by Willstätter and Mieg, to Prof. A. G. Perkin, he kindly confirmed the fact that it did not coincide with the product used by him.

† 'Journ. Genetics,' vol. 4, p. 109 (1914); and earlier papers referred to therein.

One further case needs consideration: Combes‡ states that he has isolated a crystalline anthocyan pigment from the red autumn leaves of *Ampelopsis hederacea*, whilst from green summer leaves of the same plant he obtained a yellow-brown sap-pigment in crystalline condition. He further states that, by reduction of the yellow compound, he obtained a substance (crystalline) that was identical with the anthocyan, and *vice versâ*; by oxidation of the anthocyan he obtained the yellow-brown compound in crystalline form, and identical with that obtained from the green leaves. In this case neither anthocyan nor yellow colouring matter has been identified, whilst the published data, which are scanty, leave the impression that his anthocyan may consist of crystals of a flavone derivative coloured red.

The Colouring Matters of the Viola.

At the time when the present work was commenced, our knowledge of the colouring matters of the Viola species was confined to the yellow pigments. Viola-quercitrin, from *Viola tricolor*, had been isolated and investigated by various workers, and finally proved to be quercetinrhamno-glucoside,§ whilst A. G. Perkin‖ had shown that in the ordinary Violet (*Viola odorata*) there is also present a glucoside of quercetin. It is interesting to note that the latter investigation was carried out with the ordinary violet-coloured flowers, not with white Violets (this was not specifically stated in the paper cited, but has been intimated to the author by Prof. Perkin).¶

The author proposes to examine systematically the different species of Viola, with a view to isolation and identification of both the yellow flavone, also the anthocyan pigments present in them. At the outset, self-coloured varieties were chosen, and, feeling that the anthocyan would probably give more trouble in isolation than the yellow pigment, one that contained a very large percentage of anthocyan pigment—Sutton's "Black Knight"—was first taken for investigation.

The plants from which the flowers were gathered were mostly grown for the author by Mr. Costin, of the Horticultural Department, University College, Reading, to whom, as also to Messrs. Sutton and Sons, Reading, for their interest and help, the author desires to express his thanks.

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* 'Annalen,' vol. 408, p. 147 (1915).

† 'Journ. Chem. Soc.,' 1916, p. 145.

‡ 'Compt. Rend.,' vol. 157, p. 1002; vol. 157, p. 1454.

§ A. G. Perkin, 'Journ. Chem. Soc.,' 1910, p. 1776.

‖ 'Journ. Chem. Soc.,' 1904, p. 58.

¶ The author proposes to isolate and identify the anthocyan pigment from the Violet, and thus complete the data.

The flowers were an almost dead black colour, and, when viewed by strong transmitted light, a deep purple to purple-black. The petals only were gathered, and these were air-dried, in the shade, at room-temperature, then completely dried *in vacuo* [over conc. sulphuric acid and caustic soda (solid)], powdered, and stored for use.

Contrary to anticipation, the isolation and purification of the anthocyan pigment gave but little trouble, but the method used by the author prior to the publication of the paper on the blue-black Pansy pigment by Willstätter and Weil (*loc. cit.*) was not quite so expeditious as that described by those authors.

Although Willstätter and Weil described their flowers as being blue-black, a description that could not hold for the decidedly purple-black flowers used by the present author, the data given by them concerning the pigment they obtained made it appear most probable that it was identical with that obtained by the present author.

The author anticipated obtaining a cyanidin derivative, but, having isolated a quantity of the pigment, he was prevented on account of lack of time from proceeding to the identification of the product and to the completion of the isolation and identification of the yellow pigment that had been shown to be present, and proved capable of giving rise to anthocyan by the author's reduction method, as this involved a considerable undertaking. The timely appearance of the paper by Willstätter and Weil, in which it was shown that they had obtained a delphinidin derivative, of which very clear and characteristic description was given, and which further contained data concerning delphinidin itself that greatly facilitates its identification, has enabled the author to show, with but little work and with the small quantity of pigment he had available, that his pigment is undoubtedly a derivative of delphinidin, not of cyanidin, and is without doubt identical with Willstätter and Weil's violanin. It is entirely due to the above publication that the author has been able to bring together his observations in their present form, for beyond the fact that it enabled the author so readily to identify his anthocyan pigment as a delphinidin derivative, this identification made possible some conclusions as to the yellow sap-pigment also.

If the yellow sap-pigment present is that which corresponds to the anthocyan, then, in this case, it should be a myricetin glucoside. Myricetin differs from all the other known flavonol and flavone sap-pigments in that it dissolves in dilute alkalis forming a *green* solution, which passes on exposure to air to blue, violet, then orange yellow;* moreover, in the presence of other yellow sap-pigments, a green colour passing to a brown-yellow is observed when myricetin is present. In this way its presence, together with quercetin, is shown in the colouring matter obtained from *Thuja occidentalis.* The known glucoside of myricetin, on the other hand, gives only a yellow coloration with dilute alkalis.

Now whilst the identity of quercetin, or any similar sap-pigment of the flavonol group, would have to be established by isolation and characterisation, pending time for further investigation, the presence of myricetin would be almost conclusively proved if the above-mentioned characteristic colour changes could be observed under proper conditions.

From the observations made in the course of the present investigations, and described below, there can now be no doubt that, unless gossypetin is present, which is unlikely, there is present in the Viola "Black Knight" (Sutton's), a glucoside of myricetin side by side with a glucoside of delphinidin.

Thus the presence in one flower of an anthocyan pigment and of the flavonol derivatives from which it would be produced by reduction, is shown for the first time.

EXPERIMENTAL.

Isolation of the Anthocyan Pigment of the Purple-Black Viola.

The dry powdered petals of Sutton's "Black Knight" (25 grm.) were extracted with alcoholic hydrochloric acid (200 c.c. of a mixture made up in the proportion 95 c.c. of 96-per-cent. EtOH; 5 c.c. of 2N aqueous HCl; and 1 c.c. conc. aq. HCl), and after standing some hours the extract filtered off and the residue further extracted with the same solvent. (As stated by Willstätter and Weil, MeOH may, with advantage, replace EtOH.) The combined filtrates were mixed—good agitation—with 2–2½ times their volume of ether, whereby most of the anthocyan pigment was precipitated as a sticky mass from which the liquors were readily decanted; the liquors were then filtered and worked up for the yellow pigment which they contained.

Prior to the publication of Willstätter and Weil's paper on the blue-black Pansy (*loc. cit.*), the crude anthocyan pigment—after one reprecipitation from alcoholic hydrochloric acid by means of ether—was fractionally precipitated from the same solvent by ether, then finally crystallised by solution in acidified alcohol, addition of aqueous acid (HCl) to the solution, and slow evaporation of the alcohol, as described by Willstätter and Everest, for the purification of cyanin chloride from the Cornflower.* As Willstätter and Weil's method of purification would save considerable time, it was tested with the above crude product, and found to be very satisfactory, viz.: The moist precipitate of the anthocyan pigment, either crude or preferably after one reprecipitation from alcoholic hydrochloric acid by means of ether, was dissolved in 0·04-per-cent. aqueous HCl (for the total precipitate from 25 grm. dry petals *ca.* 50 c.c. were used), the solution filtered, and the filtrate mixed with half volume of 96-per-cent. ethyl alcohol, and one volume of 4-per-cent. hydrochloric acid, then allowed to stand several days in a dish loosely covered with filter paper. Under these conditions well-formed crystals separated which were collected, washed with 7-per-cent. aqueous hydrochloric acid, and air dried (25 grm. petals gave 1·3 grm. crystals).

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* A. G. Perkins, 'Journ. Chem. Soc.,' 1899, p. 1289.

The pigment when recrystallised in this way, separated with splendid regularity as deep red, clean cut, hexagonal or tetrahedral plates.

The properties of the crystalline chloride have been carefully noted and compared with those of Willstätter and Weil's violanin chloride, and the result of the investigation leaves no doubt that the products are identical.

In view of the discovery in the alcohol-ether liquors of the presence not only of myricetin, but also of another flavone derivative which is capable of producing an anthocyan by reduction, an attempt was made to see whether any indication of the presence of other anthocyanins could be detected in the plant extract. To this end, the alcoholic hydrochloric acid extract from 1 grm. of petals was carefully fractionally precipitated by ether, the precipitate from each fraction being collected, dissolved in water, made very faintly acid, then tested in respect of the colour reaction given with ferric chloride. In all, nine fractions, which included the whole of the red pigment present, were made, all were treated in exactly the same way, and direct comparison of the colour reactions given by all nine fractions was made at the same time. No difference of any kind could be observed in the colours produced, which in every case were clear pure blue. This points to the absence of the cyanidin glucosides, cyanin, mekocyanin, idæin, asterin, and chrysanthemin, also to the absence of the glucoside derivatives of delphinidin, myrtillin, althein, the anthocyanin from *Vitis riparia*, and possibly ampelopsin, for all of these pigments produce a violet coloration under the conditions of the experiment described above, but does not preclude the presence of other anthocyanins which either give blue, or violet-blue, or do not give any coloration under the above conditions.

The quantities of material available were not sufficient to allow of further investigations on these lines.

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* 'Annalen,' vol. 401, p. 189 (1913).

In the comparison of the author's anthocyanin chloride with Willstätter and Weil's violanin chloride, the following points were examined :—crystalline form, colour and reflex of the crystalline chloride; colour of solution in aqueous acid and in alcohol; colour change on addition of ferric chloride to an aqueous, or alcoholic, solution; colour change on addition of alum to an aqueous solution; solubility in acidified methyl alcohol, in acidified ethyl alcohol, in water, in aqueous hydrochloric acid (0·15, 0·3, 0·5, 5, 20, and 30 per cent. HCl respectively), in aqueous sulphuric acid (0·05 and 7 per cent.): the distribution of pigment between amyl alcohol and dilute aqueous acid; and finally the crystalline form, colour, and solubility in water of the picrate.

A determination of the water of crystallisation carried out by Mr. A. J. Hall, B.Sc., A.I.C., showed that when dried to constant weight at the temperature of a boiling water-bath and pressure 10 mm., the air-dried crystalline chloride lost 15·68 per cent. of its weight (used 0·5658 grm. air-dried product; loss = 0·0887 grm.). Willstätter and Weil found that violanin chloride (air-dried) when dried *in vacuo* over sulphuric acid lost 15·29 and 16·15 per cent. (2 separate estimations).

Whilst the author found very slight divergences from Willstätter and Weil's descriptions in respect of colour and reflex of the crystalline chloride, which the author would describe as red-brown and golden respectively, and the solubility in very dilute (0·05 per cent.) sulphuric acid, in which it is rather more soluble than the description of Willstätter and Weil would lead one to assume, the solubility being nevertheless very small, in every other point, save the alum reaction, which was very pronouncedly different, the pigments possessed identical properties, and there can be no doubt that the pigment isolated by the author is violanin chloride. The striking difference found in the alum reaction, which at first led the author to believe that his product was different from violanin chloride, has been satisfactorily explained, as will be seen from the results below.

The Alum Reaction of Anthocyan Pigments.

In a number of instances Willstätter and his collaborators have included among the characteristic reactions of anthocyan pigments described by them, beyond a colour reaction with ferric chloride, one also with alum or, as in the case of malvin and malvidin, they have stated that pigments give neither colour reaction with ferric chloride, nor with alum.

In the case of violanin chloride, they state that alum gives a blue coloration, whereas when the author examined the pigment obtained by him from the purple-black Viola, and which is doubtless identical with violanin chloride, he was unable to obtain any such coloration.

Noting that the colour reactions of the anthocyans described by Willstätter as resulting from the addition of alum in aqueous solution were always the same as those produced by ferric chloride in aqueous solution, it occurred to the author that the reactions obtained by Willstätter with alum may possibly have been in reality the iron reaction, due to the use of impure alum containing traces of iron, whilst the author's failure to obtain the reaction with his pigment was due to the fact that, on account of the knowledge that a ferric chloride reaction was given by the pigment, an alum free from iron had been obtained for the test. To test this, a sample of commercial alum was obtained and used, whereupon the reaction was obtained exactly as anticipated, and as described by Willstätter. The presence of an exceedingly small trace of iron in the sample of alum originally used by the author was shown by adding a very considerable bulk of it to a small quantity of the solution of the pigment, when the colour reaction slowly appeared (No. 3, below).

Reagent.	When added to solution of the crystalline chloride in faintly acid aqueous solution, produced
1. Dilute solution of pure alum	No colour change.
2. Saturated solution of pure alum	Slight and very slow change towards purple.
3. Saturated solution of pure alum with addition of considerable quantity of excess solid	Slowly purple, then pure blue.
4. Dilute solution (same concentration as No. 1) of commercial alum	Very soon purple, then pure blue.
5. Dilute ferric chloride (aqueous) *ca.* 6 per cent. solution, ½ drop	Immediately through purple to pure blue.
6. Very dilute aqueous ferric chloride, 0·1 c.c., containing 0·000015 grm. ferric chloride	Very soon purple, then pure blue.
6A. Dilute aqueous solution of ferric sulphate, 2 drops	Very soon purple, then pure blue.
	When added to solution of the pigment in faintly acid ethyl alcohol—
7. Dilute ferric chloride, as No. 5	Immediately through purple to pure intense blue.
8. Dilute aqueous solution of pure alum	No colour change.
9. Dilute aqueous solution of commercial alum	No colour change.
10. Dilute aqueous solution of ferric sulphate (same solution as No. 6A), 2 drops	No colour change.
11. Solid, finely divided ferric sulphate	No colour change, even on standing, only slight change of tint towards purple.
12. Test No. 11 on dilution with water	Rapidly passes through purple to fine pure blue.

The fact that the addition of alum, containing iron, to an alcoholic solution of the colouring matter fails to give the reaction is thus shown to be due to the iron being in the form of the sulphate.

It would appear, therefore, that the colour reactions of anthocyans described by Willstätter and his collaborators as due to alum* are due to the presence of iron in their reagent, and not to the action of pure alum.

Hydrolysis of the Anthocyanin (Violanin) Chloride.

As the hydrolysis of the anthocyanin chloride had not been carried out prior to the appearance of Willstätter and Weil's paper on the blue-black Pansy, the method described by them for the hydrolysis of violanin chloride was adopted.

By carrying out the hydrolysis quantitatively, the author found that 0·4730 grm. of his anthocyanin chloride (violanin chloride), dried as described above, yielded 0·2205 grm. of anthocyanidin chloride (delphinidin chloride), dried at 100° C.; this included the small quantity recovered from the filtrate by means of amyl alcohol, *i.e.* the anthocyanidin chloride = 46·61 per cent. of the weight of dry anthocyanin chloride taken. Willstätter and Weil found, for violanin chloride, a yield of 44 and 43 per per cent. (two experiments) of delphinidin chloride—they did not recover the small portion of pigment present in the filtrate—and state that theory requires 53·85 per cent. if violanin chloride is $C_{27}H_{29}O_{15}Cl$, whereas if it is $C_{27}H_{31}O_{16}Cl$, it should yield 52·37 per cent. The exact formula is still uncertain (*cf.* Willstätter and Weil, *loc. cit.*).

The anthocyanidin chloride obtained by the author separated in minute micro-crystalline aggregates, not in the well-defined crystals obtained in the case of cyanidin chloride.

For identification purposes, the author's hydrolysis product was tested in respect of the following characteristics, viz., solubility in methyl alcohol, in ethyl alcohol, in water, in dilute sulphuric acid, and in hydrochloric acid (various concentrations); extraction from aqueous solution by ether, and removal of trace of pigment extracted by shaking with dilute acid; ferric chloride reaction, in aqueous solution, in alcoholic solution, and the effect of dilution with water in the latter case upon the coloration produced; the colour change produced when sodium carbonate is added to an acid solution; and, finally, the effect of heat upon the dry product. In every case it was found that the results obtained exactly coincided with those obtained by Willstätter and Weil (*loc. cit.*) or Willstätter and Mieg† for delphinidin chloride. The deposition of the violet colour base from the solution in water first formed by the chloride, and the absence of such deposition when warm water is added to a solution of the chloride in alcohol, which characterise delphinidin, were readily observed with the author's product.

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* *E.g.*, *cf.* 'Annalen,' vol. 408, pp. 35, 38, 50, 130, 133, 141 (1915).

† 'Annalen,' vol. 408, p. 61 (1915).

In order to confirm further the identity of the substance, a portion was converted into the picrate, which separated as bundles of fine red-brown needles, which proved to be difficultly soluble in water; this agrees with Willstätter and Mieg's description of delphinidin picrate.

A further portion was treated exactly as specified by Willstätter and Weil for the formation of their third hydrate of delphinidin chloride, and, under these conditions, the author's product readily separated in the form of beautiful brown needles, singly or in clusters, which coincided exactly with the description and illustration given by Willstätter and Weil for the third hydrate of delphinidin chloride ($C_{15}H_{11}O_7Cl,4H_2O$).

When another portion was treated as described by those authors for the production of their fourth hydrate of delphinidin chloride ($C_{15}H_{11}O_7Cl,1\frac{1}{2}H_2O$), the changes described by them were clearly observed, viz., immediate precipitation of the amorphous product, followed by gradual change, on standing, from the amorphous state into small well-defined crystals. In this case, however, the author found it necessary to keep the product somewhat above room-temperature, after the addition of the concentrated acid, in order to obtain the change from amorphous to crystalline state.

There can be no doubt that the author's anthocyanidin is identical with delphinidin.

Examination of the Yellow Sap-Pigment.

The ether-alcohol liquors from the precipitation of the anthocyan pigment were shaken with dry, precipitated calcium carbonate ($BaCO_3$ was found less satisfactory), stood for about five minutes, filtered, and the ether, then alcohol, distilled off from the liquors thus obtained.

In order to discover the colour produced by the action of alkalis on the flavone glucosides present in this concentrate, a test portion was evaporated to dryness, extracted with ether, the ethereal extract (which contains waxes in addition to flavone derivatives) washed quite free from anthocyans by means of acid, then filtered, and finally shaken with dilute sodium carbonate solution, when a clear yellow aqueous layer was formed.

The bulk of the concentrate produced by removal of the ether and alcohol from the liquors obtained from the precipitation of the anthocyan was boiled with sulphuric or hydrochloric acid, whereby the glucosides were hydrolysed, the product cooled and extracted with ether, into which the waxes and flavone derivatives passed, whilst the anthocyanidin remained almost quantitatively in the aqueous acid. The ether layer thus obtained, after separation, was washed six times with fresh quantities of aqueous acid of varying concentrations to remove all traces of anthocyan. The last four washings were quite devoid of colour, and, on making alkaline with sodium carbonate, gave but the faintest tinge of yellow. The ether layer, after receiving the above treatment, was filtered through double filter papers to remove all traces of suspended plant matter, then shaken with dilute sodium carbonate solution, whereupon a fine deep, clear olive-green aqueous layer was produced. On standing, this colour soon passed through a rather bluer shade of green to a brown-green, then to orange-yellow. These observations, together with the fact that the glucoside gave yellow, not green, with dilute alkalis, gives a strong indication of the possible presence of myricetin.

Further confirmation of the presence of a myricetin (or less probably gossypetin) glucoside in the Violas under investigation was obtained in the following way:—

When in the original precipitation of the anthocyan pigment the extract obtained from the flower petals was precipitated by means of the addition of 3½ times its volume of ether instead of 2½, the process above described gave an ether-alcohol liquor which was so completely devoid (after the $CaCO_3$ treatment) of anthocyan that its presence could be neglected. When such a liquor was freed from ether by distillation, it showed but the faintest pink coloration on acidification with conc. HCl, but, on addition of a small amount of Mg ribbon, the solution rapidly developed a fine red colour, as would be expected if a flavone derivative were present. The main bulk of the liquors were deprived of their alcohol, hydrolysed by boiling with acid, and treated exactly as described above. In this case, when the ether solution obtained was examined, it was found to contain only a flavone derivative which gave a yellow colour with dilute sodium carbonate solution, whilst, from the yellow aqueous layer so obtained, a small deposit of a yellow powder was obtained after acidification and standing. Hence there is present in the Viola under investigation not only a myricetin glucoside, but also another yellow sap-pigment, which does not give a green coloration (in sugar-free condition) with dilute alkalis.

Having failed to detect the presence of myricetin in the liquors prepared in the above manner, the crude anthocyan precipitate from which they were obtained was examined, and it was very readily shown that, by the more complete precipitation of the anthocyan pigment by use of 3½ volumes of ether in place of the 2½ previously used, the myricetin glucoside had been thrown down with the anthocyan, from which it was readily recovered.

The crude, undried, anthocyan precipitate, obtained in the above way, was dissolved in slightly warm acidified (HCl) ethyl alcohol, the solution filtered, and the anthocyan reprecipitated by the addition of 2½ times its volume of ether. The liquors were decanted, filtered through a double filter, and treated exactly as before to show the presence of myricetin.

When the washed and filtered ether solution obtained in this case—the final filtration of the washed ether extract, before testing for the colour reaction, was always included as a precaution against the possibility that a green colour may be formed as the result of some small amount of suspended matter being present—was shaken with dilute aqueous sodium carbonate solution, a splendid clear deep green was obtained that passed through the colour changes described above. In view of the fact that, as prepared in this case, the ether solution contained no waxes, and A. G. Perkin has pointed out that the colour reactions described above can only be taken as a conclusive test for the presence of myricetin (or less probably gossypetin) if it is quite certain that it is an ether-soluble substance that produces them, despite the fact that the precautions taken above appear to preclude the presence of any ether-insoluble product when the tests were made, a bulk of the ether solution prepared as in the last case was evaporated to dryness, whereby a yellow residue was obtained which also dissolved in dilute alkalis to give the colour reactions already described—green passing through bluer-green to brown-green, then orange-yellow. As a further precaution, some of the yellow residue was extracted with fresh ether, the ether extract filtered through double filter paper, and half the filtrate shaken with dilute sodium carbonate solution, when a fine green aqueous layer was obtained which passed through the same colour changes; the other half of the ether extract was evaporated, and the residue treated with dilute sodium carbonate solution, when it also produced the same colour reaction. Furthermore, the residue obtained from the bulk of ether solution originally evaporated when dissolved in acid ethyl alcohol and treated with Mg gave a clear, strong, red coloration.

These observations leave one possibility to be removed, viz., that the green coloration results from some product of decomposition of the anthocyan pigment during the hydrolysis, or from some unexplained retention of the anthocyan pigment, in colourless form, by the ether. This possibility was removed by taking 0·1 grm. of the pure crystalline anthocyanin chloride (violanin chloride)—a quantity greatly in excess of that present in the ether-alcohol liquors—dissolving it in alcohol, concentrating the solution under the conditions used for the extracts above mentioned, hydrolysing with boiling acid, then extracting the cooled product with ether, washing the ether extract, finally filtering the washed ether layer, all under exactly the same conditions as used in the examination of the yellow pigments. The ether layer thus obtained, when shaken with dilute sodium carbonate solution, gave no trace of green coloration.

In the light of the results thus far obtained, the methods employed in these preliminary investigations are capable of considerable simplification, and by taking advantage of this it is hoped that the isolation of both myricetin and the sap pigment, which gives a yellow coloration with alkalis, will be possible in sufficient quantities for confirmation of their identities by means of derivatives. It is doubtful whether the quantities present will allow of an insight into the nature of their glucosides being obtained, but an attempt will be made if it appears reasonable.

When time and circumstances permit, it is proposed to extend this work, in the first place, to various other Violas, then to *Rosa gallica* (in which the anthocyanin is cyanin), the *Pelargonium zonale* (in which the anthocyanin is pelargonin), and other flowers, with a view to a systematic investigation on the lines developed in the present paper, and in suitable cases the examination of the glucoside pigments with a view to discovering whether, where the anthocyan pigments are accompanied by corresponding flavone derivatives, the two classes of pigment occur attached to the same sugars.

Having proved that the anthocyanin described in the present paper is identical with Willstätter and Weil's violanin, it is not the author's intention to encroach upon the further work outlined by them (*loc. cit.*) concerning the details of its constitution.

Note on the Nature of Growths in Colloidal Silica Solutions.

By H. Onslow, Trinity College, Cambridge.

(Communicated by Professor F. G. Hopkins, F.R.S. Received March 7, 1918.)

The late Dr. Charlton Bastian,* after having performed a number of experiments, claimed to have synthesised certain symmetrical bodies resembling torulæ and other minute organisms, from sterilised colloidal solutions which had been exposed for a long period to the light. Further, he claimed that such "organisms" were capable of reproducing themselves.

In spite of the inherent improbability of these results, Dr. Bastian was so insistent in his claims, and so anxious for independent investigation, that I undertook to repeat his experiments carefully, in order to ascertain whether the organised bodies in question were in reality living protoplasm.

The difficulty of proving a negative is obvious, but it is hoped that the following experiments, taken in conjunction with recent work† in the same field, may help to decide whether the forms observed by Dr. Bastian were, as I now believe, colloidal simulacra, or no.

The existence of living organisms can be most conclusively proved by subcultivation in media that have previously been shown to be sterile. The procedure adopted was therefore based on this principle. The media used consisted of: (1) Dr. Bastian's neutral ammonium tartrate and sodium phosphate solution;‡ (2) ordinary sterile nutrient broth; and (3) "tryptic broth," a special medium recently described by S. W. Cole and the author.§ This medium is prepared from a solution of casein digested with trypsin, and was adopted after Dr. Bastian had stated|| that the addition of a trace of tyrosine greatly increased the number and the rate of growth of the organisms, as well as the positive results. "Tryptic broth" contains not only a considerable amount of tyrosine, but of other amino-acids as well, and is therefore particularly favourable to the growth of micro-organisms.

A series of 90 tubes was prepared, using three different samples of

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* 'The Origin of Life, etc.,' by H. Charlton Bastian, F.R.S.

† B. Moore and J. A. Webster, 'Roy. Soc. Proc.,' B 593, p. 163 (October, 1913); B. Moore, 'Roy. Soc. Proc.,' B 609, p. 27 (July, 1915).

‡ 'The Origin of Life' (2nd edit.), by Dr. Charlton Bastian, p. 40.

§ 'Lancet,' July 1, 1916.

|| 'Nature,' p. 537, July 15, 1915.

sodium silicate. Since there is some doubt as to the stability of solutions of this substance, precautions were always taken to prepare them from the stock samples immediately before use. The tubes employed* were the same as those made for Dr. Bastian. They came sealed, but were always heated before use for an hour at 200° C. When cool the tips were broken off, and the tubes half filled with the appropriate solutions. After re-sealing they were sterilised either for (1) 10 minutes at various temperatures between 110° and 130° C., or (2) 60 minutes at 100° C., or (3) 20 minutes at 100° C., on three successive days. Sterilisation was carried out either in the autoclave, or, following the method employed by Dr. Bastian, in a calcium chloride bath. After sterilisation they had the colour and appearance described by Dr. Bastian, and no doubt contained solutions of ferric and silicic oxides. The tubes were usually kept in the incubator at 37° C. for at least one month, and then in subdued daylight for various periods up to three years, before examination. This was carried out by the microscopical observation of the centrifugalised deposits with a $\frac{1}{6}$ inch objective and by sub-cultivation. To avoid accidental contamination, a square tent was made by hanging up cloths steeped in 2-per-cent. lysol, within which all subsequent operations were carried out. As an additional precaution, the air was sometimes sprayed by means of a steam atomiser, charged with 4-per-cent. lysol, to remove particles of dust, etc.

Sterilised pipettes, which had been inserted through the plugs of test-tubes, were prepared, as well as tubes of sterile "tryptic broth." The tubes containing the experimental solutions were thoroughly shaken, to remove any particles adhering to the walls, centrifuged, and allowed to stand for a day or two. Their necks were then ringed with a glass-cutting knife, and, after all the tubes, as well as the hands, bench, etc., had been moistened with lysol, their necks were removed by means of a white-hot point of glass. Most of the precipitate, and about 2 c.c. of fluid, were then removed to the culture medium by means of a fresh sterile pipette, care being taken to flame the mouths of both tubes. The broth tubes were first incubated for 10–14 days at room temperature, and then for an equivalent period at 37° C.

The solutions were made according to Dr. Bastian's directions.†

The "colourless" solution contained: ammonium phosphate, 6 grains; dilute phosphoric acid, B.P., 6 drops; sodium silicate (dilute), 2–10 drops.

The "yellow" solution contained, in addition to the above, eight drops of liquor ferri pernitratis, B.P. Since Dr. Bastian attached the utmost importance to the particular sample of silica used, I employed the three following:—

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* Procured from Müller, Orme and Co., 148, High Holborn, London, W.C.

† 'Origin of Life,' by Dr. Charlton Bastian, pp. 30 and 90–91.

Sample I.—Bought (1913) from Allen and Hanbury, reserved for Dr. Bastian since 1910, and recommended by him ("Origin of Life," p. 29, 2nd Ed., 1912).

Sample II.—0·01 per cent. colloidal solution, specially prepared by Grübler, Leipsic, in 1910, and used immediately.

Sample III.—Sp. gr. 75°. Bought (1914) from Allen and Hanbury and recommended by Dr. Bastian.*

In a private letter Dr. Bastian recommended three drops of this last sample for the "colourless solution" and five drops for the "yellow solution." In a later article, however,† he said that it was not satisfactory in the quantities previously mentioned, but successful in larger quantities and with other reagents. The amount of Samples I and III used in my experiments was from 2 to 5 drops of a solution consisting of equal volumes of sodium silicate and distilled water, and of Sample II from 10 to 12 drops.

The results obtained with all the 10 sets of tubes prepared may be illustrated by the three following examples:—

Series.	No. of tubes.	Contents.	Sterilisation.	Period exposed to the light, in months.	No. of tubes.	Remarks.
A	10	"Yellow solution," Sample I, 3 drops.	10 minutes at 130° C.	4½ 30	3 7	A few dead bacilli were found in some tubes, and in others there were numerous oval bodies about the size and appearance of torulæ.
B′	10	"Yellow solution," Sample I, 2 drops.	10 minutes at 110° C.	2½ 35 Lost	2 4 4	In one tube alone living bacilli were found. They caused a thick pellicle on broth, grew in chains, and resembled *B. subtilis*. Easily subcultivated on all media.
H	10	"Colourless solution," Sample II, 12 drops.	20 minutes at 100° C. on 3 successive days.	18¾ 26 33½	1 1 8	A few dead bacilli were found with square ends. It was impossible to subcultivate them on any of the three media used.

It will be noticed that a square-ended bacillus, somewhat like *B. subtilis* in appearance, was on several occasions found in the tubes, but in every tube but one (from Series B′) they were dead and incapable of being sub-cultivated. I am well acquainted with the morphology of this organism and with some

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* 'Nature,' January 22, 1914.

† *Ibid.*, December 24, 1914.

PROCEEDINGS
OF
THE ROYAL SOCIETY.

Series B. Vol. 90. **No. B 629.**

BIOLOGICAL SCIENCES.

CONTENTS.

Page

Brevity, Frequency of Rhythm, and Amount of Reflex Nervous Discharge, as indicated by Reflex Contractions. By N. B. DREYER and C. S. SHERRINGTON, F.R.S. 270

CROONIAN LECTURE.—The Physiological Basis of Thirst. By Major WALTER R. CANNON, M.R.C., U.S. Army; George Higginson Professor of Physiology, Harvard University 283

The Mechanism and Control of Fibrillation in the Mammalian Heart. By J. A. MACWILLIAM, F.R.S. 302

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PRINTED AND PUBLISHED FOR THE ROYAL SOCIETY BY
HARRISON & SONS, 44—47, ST. MARTIN'S LANE, LONDON, W.C. 2.
Price Three Shillings.

No. B 629.

October 17, 1918.

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of its cultural characteristics, as it exists abundantly in my laboratory and is a frequent source of contamination. In one tube only was this organism found alive, that is to say, capable of sub-cultivation. As a matter of fact this tube was opened soon after it had been sterilised, together with one or two others from each set to serve as controls in the event of any positive results occurring, and unfortunately without the stringent precautions used in the case of the other tubes. There can be no doubt that the presence of the organism was attributable to contamination from the air during sub-cultivation, as the tube had only been exposed to the light two months, and therefore could not have grown even in the way described by Dr. Bastian.

The dead bacilli of similar appearance found in a few other cases may be accounted for by their unavoidable introduction into the tubes before sterilisation. In many of the films spread directly from the precipitates more or less circular bodies were to be seen, which roughly suggested torulæ. In one tube (Series A) they were especially regular and life-like, but in other tubes they varied in size and shape from specimens which were indistinguishable from torulæ to those which were obviously irregular plates of silica, which readily stain in the usual way.

I am convinced that the method employed yields tubes which are absolutely sterile even after three years have elapsed. I am, therefore, forced to the conclusion that the bodies found by Dr. Bastian, which often resembled living organisms in a striking fashion, are due to the slow deposition of silica from the colloidal solutions, either about minute nuclei or about the detritus of dead organisms, in the manner described by Profs. Moore and Evans,* and also by Sydney G. Paine.† The only living organism which occurred in my experiments must, therefore, have been introduced accidentally owing to the lack of sufficient precautions. For when great care was taken to avoid contamination the tubes were found to be uniformly sterile.

I am at a loss to account for the results of A. and A. Mary‡ (whose original communication I have been unable to consult owing to present circumstances), unless they also are due to accidental contamination.

In conclusion I would like to say that Dr. Bastian was at all times most courteous and kind in giving me information, as well as in allowing me to see his slides.

I am also greatly indebted to Prof. F. G. Hopkins for kindly revising this paper.

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* 'Roy. Soc. Proc.,' B, vol. 89, p. 17 (1915).

† 'Annals of Botany,' vol. 30, No. 119, p. 383 (July, 1916).

‡ 'Le Médecin,' Brussels, October 31, 1913, and January 15, 1914; 'Knowledge,' January–September, 1917, p. 7.

Brevity, Frequency of Rhythm, and Amount of Reflex Nervous Discharge, as indicated by Reflex Contraction.

By N. B. Dreyer and C. S. Sherrington, F.R.S.

(Received May 31, 1918.)

During an enquiry, for Surgeon-General Sir David Bruce's Committee on Tetanus, into the neuro-muscular changes produced by tetanus-toxin, it became desirable to re-examine certain commonly accepted data regarding the reactions of normal reflex-centres.

I.

The first of these that engaged us regarded the reaction of a spinal reflex-centre to a single momentary stimulus applied to a main afferent nerve playing on the centre. Evidence was desired as to whether the reflex nervous discharge in response to the single volley of simultaneous centripetal impulses thus thrown into it consists similarly of a single volley of emitted impulses, or whether it consists of a volley-series, brief but yet repetitive, a series of volleys. The form of the reflex contraction should throw light on this, when compared as to its space and time relations with that of the simple muscle-twitch evoked by a like stimulus applied to the motor nerve of the same muscle as that observed for the reflex.

That in certain cases the reflex response is a single-volley discharge is extremely probable. The proprioceptive reflex of vastocrureus ("knee-jerk")* and of tibialis anticus† appears to be simple twitches commonly though not always. Using the same reflex as we ourselves turned to, Forbes and Gregg‡ find that as evoked by a single break-shock the reflex nervous response, as judged by its electrical effect, is often a single-volley discharge; they point out that dicrotism of the response need not mean that any neurone discharges twice. On the other hand, the fact that,§ when the single break-shock is strong, the reflex contraction may exceed in height the maximal twitch obtainable direct through motor nerve suggests that the reflex discharge is then, although brief, a repetitive volley-series. Beritoff|| notes that in reflex tetani produced in the winter frog by strong faradisation at rates below 40 per second, some of the component shocks used as

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* *Cf.* W. A. Jolly, 'Quart. Journ. Exp. Physiol.,' vol. 4, p. 66 (1911).

† C. Asayama, 'Quart. Journ. Exp. Physiol.,' vol. 9, p. 265 (1915).

‡ A. Forbes and A. Gregg, 'Amer. Journ. Physiol.,' vol. 37, p. 175 (1915).

§ Sherrington and Sowton, 'Journ. Physiol.,' vol. 49, p. 331 (1915).

|| J. S. Beritoff, 'Zeitschr. f. Biol.,' vol. 62, p. 125 (1913).

stimuli may be followed by as many as 3–5 muscular action-current waves. The well-known slow subsidence of contraction frequently met as the after effect (T. Graham-Brown's* terminal phenomenon) seems an instance of the same kind, though more pronounced.

For our observations the muscle used was tibialis anticus, the preparation a spinal cat previously decerebrated under chloroform, the afferent nerve the popliteal, and the stimulus a single break-shock obtained by opening the primary circuit by a pendulum key set similarly throughout the whole series of experiments. The muscular contraction was recorded by an isometric myograph whose own vibration period was much shorter than that of the simple muscular twitch and damped to give it some "dead-beat" character. After the samples of reflex contraction had been obtained by stimulation of the popliteal nerve, the peroneal nerve, innervating tibialis anticus, was severed and the stimulus, arranged to be maximal, was applied to the distal stump of this motor nerve itself. The break-shock cathode was placed proximal on the afferent nerve for the reflex, distal on the motor nerve for the "direct" twitch. The preparation and its attachment to the myograph remained unaltered. The interval between the two observations was sometimes only four minutes, the electrodes and interelectrodal distance remained the same for both. Contraction curves showing duration and tension-height concurrently were thus obtained for the same muscle both in its reflex and direct response in the same individual preparation using a single break-shock stimulus delivered by the same key.

The condition of the preparations was usually very good, judging from the lowness of the threshold stimulus, both reflex and peripheral. It is to be regretted that the stimulus value was not obtained by the Martin† method, but the apparatus for that has not been at hand for us. The values taken were readings of the Berne inductorium scale, the primary fed by a 2-volt cell. The threshold readings were often very low indeed, far below 30 Berne units (=27 cm. on the ordinary scale), *e.g.*, 30 units—70° angular turn of the secondary spiral, sometimes 30°–80°. Often the difference between the threshold for the reflex and for the motor-nerve observation was only a few degrees of the angular scale.

Results.—Our graphic records of the reflex contraction to the single break-shock have not rarely been very similar to those of the twitch-contraction to the shock applied to motor nerve itself. This has been so most frequently when the break-shock stimulus for the reflex is near threshold value; it is the case also sometimes when the break-shock stimulus is much above threshold. More usually, however, the reflex contraction is more prolonged, and commonly also of greater height, which with the isometric torsion myograph means of greater power, than is the maximal twitch evoked by a single break-shock applied to motor nerve. This is sometimes so when the break-shock stimulus used is not far above threshold value; it is almost always so, in our experience, when the stimulus, though still of moderate intensity, is yet considerably above threshold value. The same stimulus, as judged by position of the secondary coil on the inductory scale, gives reflexly a contraction more powerful and more prolonged than it gives when applied to the motor nerve itself. At greater strengths still the single-induction shock produces reflexly a contraction which may have a height thrice as great and a duration seven or eight times as long as the maximal contraction which it produced when applied directly to the motor nerve itself. There can thus be no doubt but that a single momentary stimulus applied to an afferent nerve often evokes a repetitive discharge of impulses from the reflex centre to which that afferent nerve conducts. The

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* T. Graham-Brown, 'Quart. Journ. Exp. Physiol.,' vol. 7, p. 199 (1914).

† E. G. Martin, 'Measurement of Induction Shocks,' New York, 1912.

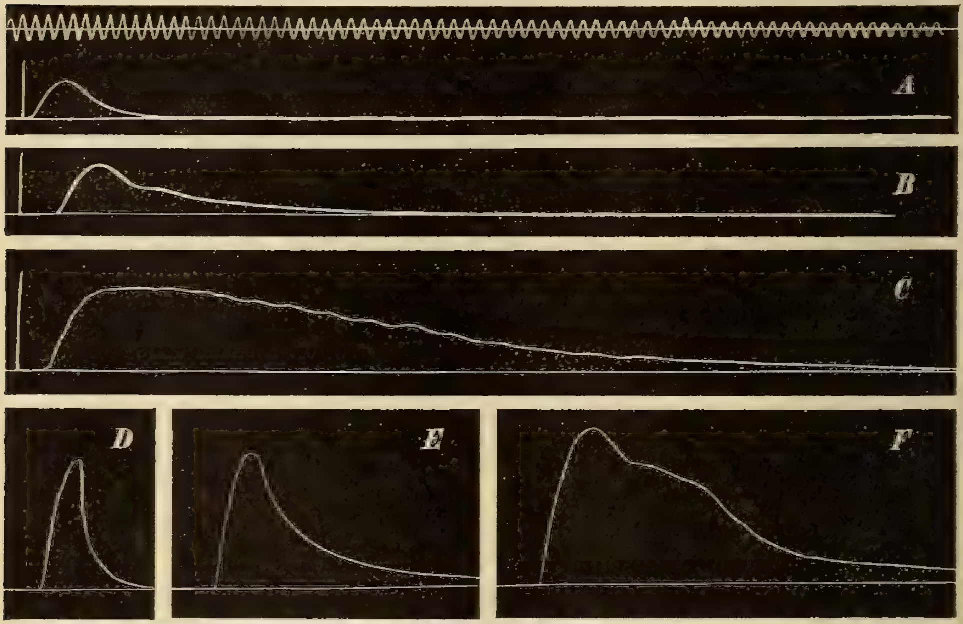

Fig. 1.—Contractions of *tibialis anticus* (spinal cat) in response to a single break-shock stimulus, in B, C, E, F, applied to afferent nerve (popliteal), in A and D applied to motor nerve (peroneal) direct. A, B, C, from one preparation; D, E, F, from another preparation. A and C, break-shock = 1000 units of Berne coil = 14 cm. distance on scale; B, at 500 units = 15·8 cm. on scale; D and F, at 15 cm. on scale = 750 units; E, at 18 cm. on scale = 200 units. Top line, tuning-fork marking 0·01 sec.

repetitive discharge occurs not unfrequently when the stimulus is not strong. Condition of the reflex centre rather than intensity of stimulus is the decisive factor.

II.

A second point engaging us concerned the frequency of the rate of the impulse-volleys of the reflex discharge in response to rhythmic stimulation of the afferent nerve at various rates of frequency. A conclusion drawn by previous observers* has been that, when the spinal centre receives successive stimuli at rates of frequency greater than 10 a second, the motor centre ceases to follow the rhythm of the stimulation. Its response is said to be then of an independent rhythm rate, a rhythm proper to itself and exhibiting a frequency of about 10 a second. The basis of this conclusion was the observation that, below rates of stimulation of 10 a second, the muscular contraction showed waves synchronous with the rhythm of stimulation of the reflex channel, and that, with stimulations more frequent than 10 a second, the muscular contraction exhibited, instead of undulations synchronous with the stimulus rhythm, undulations, slight and somewhat irregular, recurring at the rate approximately of 10 a second. This observation and conclusion were at variance with some earlier results by François Franck and Pitres† ; they were difficult to harmonise with certain experience of our own in nerve-centres poisoned with tetanus-toxin. Hence the necessity for re-examining the point in normal reflexes in order to confirm or not its normal occurrence. For this our experiments have been with the flexion-reflex of the hind-limb (cat), the preparation being spinal, the popliteal trunk the afferent nerve, and the muscle tibialis anticus, the main flexor of ankle.

(1) The contraction was recorded isometrically, the myograph recorder having a vibration period of about 0·011 second. In the primary circuit of the inductorium was a flat spring, whose vibration frequency, by alteration of the length of the spring, could be varied readily between 10 and 60 a second. The spring was armed with a fine style set close above a mercury pool. We find the myograph of the reflex contraction exhibits clear mechanical rhythm synchronous with the stimulation at rates up to 55 a second, and sometimes beyond that. With rates up to 30 a second the synchronous tremor is so coarse as to be obvious to the unaided eye. This method of synchronous rhythm showed, therefore, in its graphic application, that at frequency-rates up to somewhat above 55 per second, the rhythmic

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* V. Horsley and E. A. Schäfer, 'Journ. Physiol.,' vol. 7, p. 111 (1886).

† F. Franck and A. Pitres, 'Archives de Physiol.,' Ser. 3, vol. 5, p. 18 (1885).

discharge of the reflex centre follows the full frequence-rate of the afferent nerve stimulation, the centre emitting successive volleys of centrifugal impulses at the same rate as those evoked in and transmitted to it by the afferent nerve.

(2) The finger, on touching the muscle-tendon, detects a slight thrill at rates of stimulation of the afferent nerve even above 55 per second. To test whether this thrill has the same frequency as the stimulation rate, a myograph recorder was prepared, whose vibration period was 65 per second when attached to the contracted muscle, as tested by suddenly jerking and releasing it. The afferent nerve was then stimulated with a break-shock series of 65 per second. The recording myograph was thus used as a resonator for that pitch of vibration which should obtain in the muscle under the stimulation used if the reflex harmonised with the afferent nerve's stimulation. The result was that the myograph record then exhibited a vibration of 65 per second throughout the duration of each reflex tetanus.

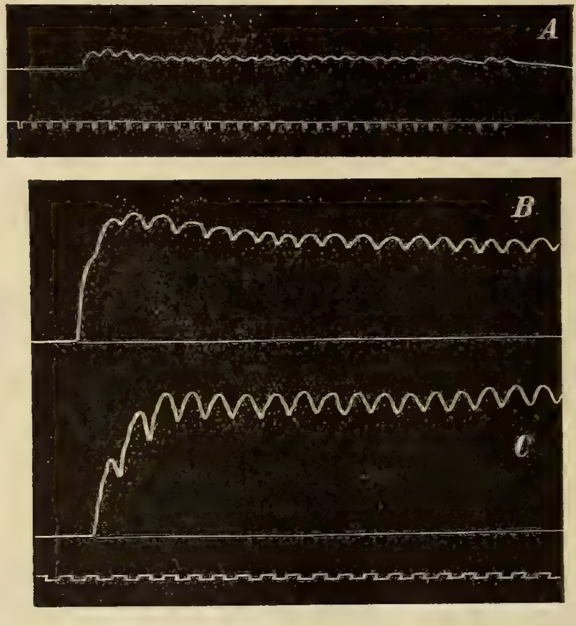

Fig. 2.—A, reflex contraction of *tibialis anticus* (spinal cat) to faradisation of afferent nerve (popliteal), at 15 per sec., as signalled below. The period of unshortcircuiting the second circuit is not shown, but the interrupter in primary circuit lapsed during the time of unshortcircuiting, and just prior to that one of the interruptions missed, and this irregularity the reflex tetanus has followed perfectly. B and C, similar preparation from another experiment; faradisation at 23 per sec.; B, the reflex response; C, response to stimulation of motor nerve; second coil at 18 cm. = 250 Berne units, for both.

Various strengths of stimulus were used, and various heights of tetanic contraction resulted, and all showed fine tremor of 65-per-second frequency. The same "resonance" method, when applied to tetani similarly provoked, but through the motor nerve direct, instead of through the afferent nerve and reflex centre, similarly gave tremor of 65-per-second rate, somewhat more marked in degree than with the reflex tetani. A resonance method is obviously open to some fallacies. Any movement of the recorder is liable to be accompanied by undulations of the periodicity proper to the recorder. But the continuance of the vibration throughout the long flat top of each tetanus record argues that underlying that there was a muscle vibration with which the lever's own period truly synchronised.

(3) The applicability both of the first mentioned "synchronous rhythm" method and the "resonance" method, for the purpose in view, depends obviously on two conditions. The individual centrifugal impulses composing each volley must be very approximately simultaneous; if they are not so, the end of one volley will commingle with the beginning of the next, and, the muscle contraction examined being that of its fibre-complex as a whole, the contraction waves will merge and may become inseparable in the record. Also, even though the successive centrifugal impulse-volleys remain discrete, the sluggishness of the contraction wave itself sets a limit to the separateness of successive waves when they follow at rates higher than a certain number per second, which number may conceivably be much lower than that at which the reflex centre can still discharge discrete impulse volleys. This limitation, due to the relatively prolonged apex-maximum of each contraction wave, can be determined by recording the tetanic contraction of the muscle

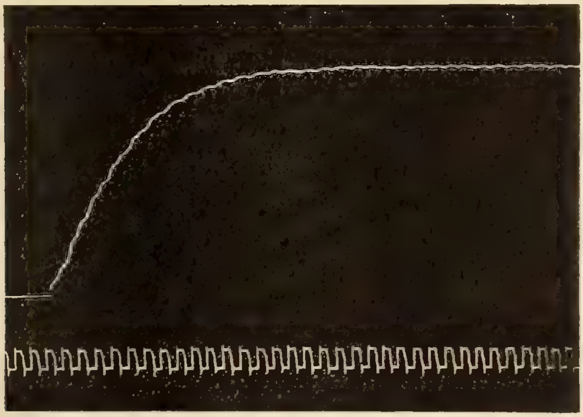

FIG. 3.—Reflex response of *tibialis anticus* (spinal cat) to faradisation of afferent nerve (popliteal) at 60 per sec.

in response to repetitive stimulation of the motor nerve at various rates. We have controlled this in the muscle we used for the reflex, tibialis anticus, and in the gastrocnemius (without soleus), and find the limit with our myograph at close to 65 per second. Above that frequency, and sometimes a little below it, the tetanic contraction in response to motor-nerve stimulation exhibits practically no visible undulation.

It was noted, however, by Marey,* a number of years ago (1867), that when the motor nerve is stimulated by rhythmic stimuli of progressively quicker and quicker rate there is, after a frequency has been reached at which the tetanic contraction becomes a smooth non-vibratory line, a still further increase in the contraction height and tension on further increasing the frequency of the rhythmic stimuli. " Après que toute vibration a disparu dans le graphique, on voit la ligne tracée s'élever de plus en plus sous l'influence d'excitations de plus en plus rapprochées."† This further increment of contraction, due to increment in number of nerve-impulses per second in the motor nerve, offered a means of testing whether the reflex centre can respond with increased frequency-rate of discharge even after its afferent nerve's stimulation-rate has reached and passed beyond that pitch at which in the muscle individual contraction waves cease to be visibly distinguishable by the myogram.

Marey's observations, in the description extant of them, may not have been entirely free from objection; using a rotating interrupter, as he did, the individual breaks are more sudden under the quicker rotation, and the individual break-shocks therefore become more powerful as individual stimuli. He was, however, presumably using maximal stimuli throughout, and in that case further increase in their stimulation potency would not account for the increase of contraction observed. In applying the experiment to reflex contraction, where it is less easy to be sure that the individual stimuli are maximal, it would be preferable, when increasing their serial frequence, not to change the speed of the individual acts of breaking of the primary circuit.

To attain this, the following plan was devised (C. S. S.). The current path of the primary circuit was bifurcated for a short distance into two equal branches, each including a Hg pool, the twin pools lying under the ends of a horizontal spar, which was fixed transversely at its middle to a horizontal steel wire stretched between rigid uprights. The wire carried a horizontal armature, which could be acted on by the poles of a small electromagnet, and from one end of the armature a vertical needle on slightly torsing the stretched wire dipped into a Hg pool beneath it. An electric circuit, independent of the stimulating circuit, was arranged so that its current passed through the electromagnet when the armature needle entered the Hg pool beneath. The wire therefore continued to vibrate at its own torsional frequency, the rate depending on its length and diameter and on the weight and moment of the armature and cross-spar with which it was loaded. The cross-spar carried, insulated from the torsion-vibrator's circuit, two fine wires re-uniting the short twin paths of the primary circuit of the stimulating inductorium. Each of these wires joined at its one end a fine gilt needle fixed vertically at end of the horizontal spar, the needle point lying close above the corresponding Hg pool. When the steel wire was torsed, one of the twin branches of the primary circuit was made and the other broken, and *vice versâ* in its elastic recoil in the opposite direction. With both of the twin branches of the circuit in use, the current passed therefore alternately by one and the other, the speed of break (and make) being identical in the two. With both of the twin branches in use, the frequency of breaking (and making) of the primary circuit was, of course, the double of the frequency when only one of them was in use. The frequency of the torsion vibration could be readily altered within wide limits by altering the length of the steel wire or by adjusting a screw-weight attached to the armature carried by the wire.

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* E. J. Marey, 'Du Mouvement dans les Fonctions de la Vie,' p. 376. Paris, 1868.

† *Loc. cit.*, p. 376.

With this interrupter the frequency of the break-shocks applied to the nerve could, by closing a key, be doubled and, by opening it, halved without other change in the stimulation as it progressed; we sought for the degree of frequency beyond which further increase of frequency, *e.g.* doubling, caused no further appreciable increase in the tetanic contraction. Using it on the motor-nerve direct we confirmed Marey's observation that further increase of stimulus frequency beyond that at which the myograph record becomes steady and non-vibratory does produce increase in height and tension of contraction up to a certain point.

Turning then to the reflex preparation, and using similar reflex and similar isometric records to those already mentioned our results have been as follows. When the reflex contraction was in progress under a stimulation of the afferent nerve at 50 per second, increase, namely doubling, the frequency caused immediate marked increase in the height (tension) of the contraction, which fell back to the previous height again at once on returning to the previous slower rate. There was no reason to suspect any difference between the making and breaking of the primary circuit as carried out by the torsional interrupter in the two twin branches respectively. But to control this as a possibility the observations were repeated with alternate priority of one or other of the interrupted branches. It made no difference to the result which one of the two branches of the circuit preceded in the sequence.

A control of this point lay also in measurement of the threshold stimulus for contraction as evoked by the circuit through each of its twin paths respectively; the threshold was found to be the same for both. A further control was obtained by comparing the height of the reflex tetanus produced (α) by use of both paths, in the way above described, with the tetanus height produced (β) by use of either of the paths alone, *i.e.*, half the frequency rate of α, the secondary coil being set further from the primary coil for obs. α than for obs. β, so that the strength of the individual shocks was less for the doubled frequency-rate than for the single-path frequency-rate. It was found that the strength of the individual shock might be considerably less during the doubled-frequency rate than during the undoubled, and the resulting tetanus height yet be greater for the doubled than for the undoubled, although the frequency-rate of the latter was 50 per second or over. Thus, in instance, a shock-series at 110 per second, the secondary coil being at 100 units on the Berne scale (secondary coil 20·3 cm. from primary), gave a tetanus record of 14 mm. height; while in the same preparation the two immediately preceding observations with either of the twin paths singly gave a tetanus of 12 mm. height only, although for them the secondary stood at 150 units on the Berne scale (secondary coil 19·4 cm. from primary), the frequency-rate of each of the twin paths being, of course, 55 per second.

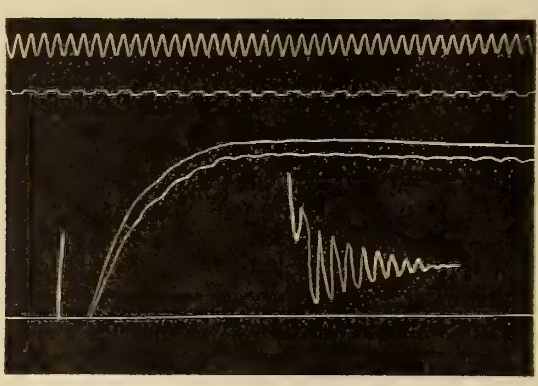

FIG. 4.—Two reflex responses of *tibialis anticus* (spinal cat) obtained in immediate succession, the lower to faradisation (of afferent nerve, popliteal) at 54 per sec., the higher by doubling the frequency (see text); the higher with the coil at 15 cm. = 750 Berne units, the lower with coil at 14·5 cm. = about 850 Berne units. Top line, 100 per sec. fork; second line, signal for 55 per sec. interrupter. Inset: the record of the vibration proper to the recording spring, lever, and attached tendon.

The individual break-shock stimuli being similar throughout and delivered throughout by the same unmoved electrode (cathode for break-shock proximal to anode on the afferent nerve), the sole stimulation-change accompanying the contraction-increment is the increased number of stimuli per second. This increase of contraction-height on increasing (doubling) the stimulation frequence was observed in experiments carrying the initial, *i.e.*, undoubled, frequence of stimulation up to 75 per second. When that rate was reached the increment of contraction-height obtained by further increase (doubling) became quite small, though still quite indubitable. The rate of

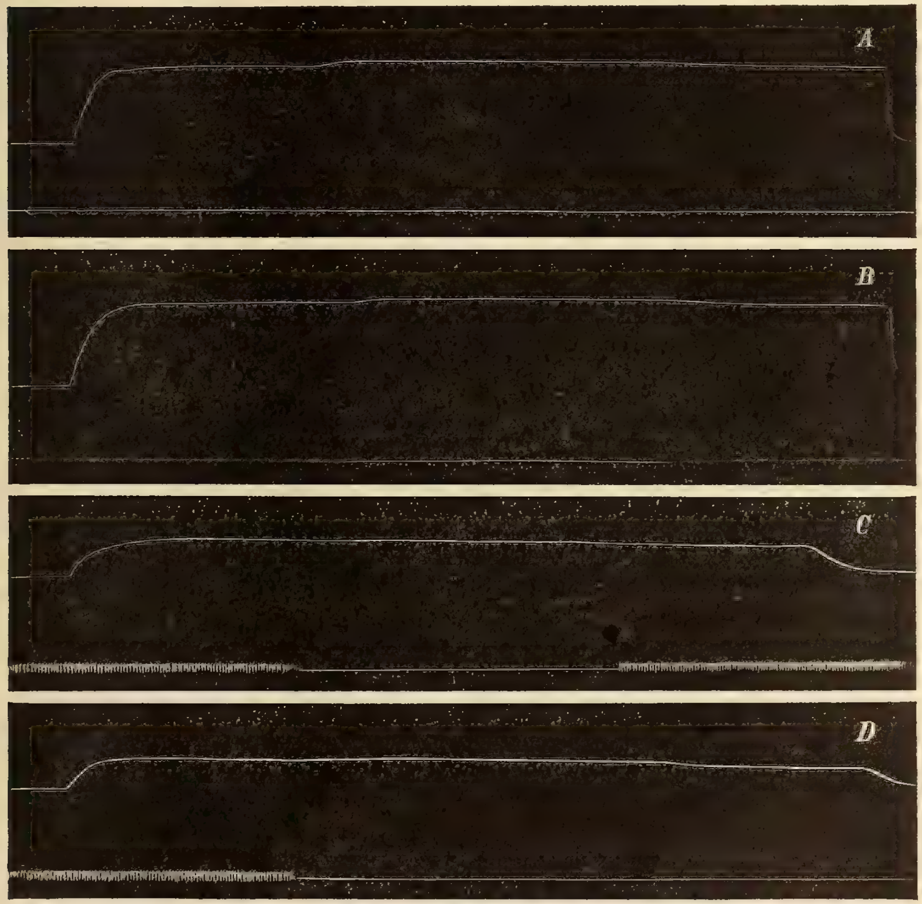

FIG. 5.—Reflex contractions of *tibialis anticus* (spinal cat), showing effect of intercurrent doubling of frequency rate of interrupter in primary. A and B, initial (and terminal) rate of stimulation, 65 per sec.; intermediate period, shown by higher plateau of contraction, at 130 per sec. In B the order of succession of the twin branches of the circuit is reverse of that in A. C and D similar, but with rate of 75 per sec. (doubling to 150 per sec.); the increase of height of the plateau obtained by doubling is still obvious, though less than in A and B. The signal, as set for the slower rate of interruption, has failed to pick up the quicker rate.

reflex discharge must, therefore, with increase of the rate of stimulation of the afferent nerve to beyond 75 per second increase to beyond 75 per second. Whether that rate approaches actually near to the limit of the rate of frequency which the reflex centre's discharge can follow cannot, of course, be answered by this method. There is nothing to show that when in the experiment the stimulation-frequency is changed from 75 per second to 150 per second, the reflex discharge does not, in fact, follow the latter figure, as we may suppose the motor nerve to do in the case of its direct stimulation.

The results by mechanical methods stand therefore no longer in contradic- with those obtained by the galvanometer using oscillations traceable to muscular action currents. C. Foà* followed in the chloralised dog action currents of the contracting femoral quadriceps synchronous with the electrical stimuli up to 20 per second, and in the frog up to 58 per second. Beritoff† concludes from galvanometric records that the discharge-frequency of the flexion-reflex in the winter-frog may approach to 75 per second, and in the summer frog to 150 per second, and P. Hoffmann‡ that in the frog the frequency may follow at first at 100 per second, though soon dropping to half that number. With these our observations by mechanical registration in the "spinal" cat are obviously perfectly compatible.

The datum that the spinal reflex-centre is a mechanism constructed with a recurrent refractory phase of 100σ duration was irreconcilable with observations§ tending to show that the duration of the spinal reflex-centre's refractory phase is of an order not far removed from that of the nerve-muscle preparation itself. The present results greatly relieve that difficulty, for, although their method does not measure the actual duration of the spinal refractory phase, they do show that the extremest length of that phase does not, under ordinary circumstances, extend beyond 12σ.

III.

A third point requiring re-determination was the ratio between the maximal power of the reflex tetanus and that of the peripheral tetanus excited by faradisation of the muscle's motor nerve. The statement based on direct experiment has been that the maximal power of the spinal reflex contraction amounts to less than six-tenths of that developed by the maximal contraction evoked by direct faradisation of the motor nerve.||

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* 'Zeitschr. f. allg. Physiol.,' vol. 13, p. 35 (1912).

† 'Zeitschr. f. Biol.,' vol. 62, p. 125 (1913); *ibid.*, vol. 64, p. 161 (1914).

‡ 'Archiv f. Physiol.,' 1911, Suppl., p. 233.

§ Sherrington and S. C. M. Sowton, 'Journ. Physiol.,' vol. 49, p. 342 (1915).

|| V. Horsley, 'Brain,' vol. 21, p. 547 (1898).

For examining this point, we have again used tibialis anticus as muscle, and as our spinal reflex the reflex evoked by faradisation of the popliteal nerve, and then in the same preparation we have registered similarly the maximal tetanus obtainable by direct faradisation of the motor nerve (peroneal) itself. We employed the same frequency-rate of faradisation for both reflex and direct effect, and both observations were obtained with exactly the same registering apparatus, sometimes within four minutes of each other. The myograph resistance was, as in our other observations, a torsion-wire and the record isometric. The upper part of the scale of resistance of the myograph was, within the limits of pull developed by the muscular contraction, almost as open for the higher limits as for its range near zero. The animals (cat) were young and small, and the maximal tensions in different animals observed varied between 650 and 940 grm.

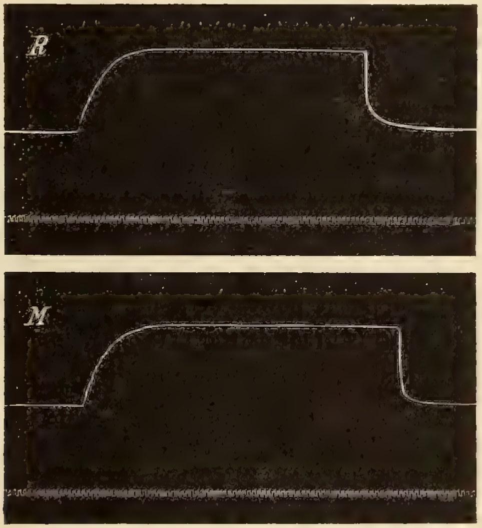

Fig. 6.—Maximal reflex (R) and maximal motor-nerve (M) responses of *tibialis anticus* (spinal cat) obtained from same preparation, rate of faradisation 75 per sec. in each case, and the secondary coil being at 12·8 cm. = 2000 Berne units for both. Under each the signal recording the interruptions in the primary.

Our results have been as follows: The ratio between the power of the maximal tetanus of the spinal reflex and that of the maximal tetanus obtainable by faradisation of the motor nerve is often unity, 1/1. In not a few preparations it is somewhat less, *e.g.*, reflex/direct = 9/10; in some preparations it is very markedly less, *e.g.*, 6·5/10.

That the maximal power of tetanic contraction obtainable from a muscle reflex is often equal to the maximal tetanic power obtainable, under the same frequency-rate of faradisation, by direct faradisation of the motor nerve itself, bears on the question whether any one afferent nerve can set into action the whole of the reflex centre to which it is afferent.*

Summary of Conclusions.

1. A single momentary stimulus of moderate intensity, *e.g.*, a break-shock, even though not far above threshold value of stimulation, applied to the afferent nerve of a spinal reflex centre, evokes from that centre not uncommonly a brief repetitive series of volleys of motor impulses. This it tends to do the more the stimulus, within limits, is increased in intensity, but the state of the reflex centre at the time is also a decisive factor.

2. The rhythm of repetition of volley-discharges from the spinal reflex centre is traced by the mechanical method to be of synchronous rate with that of stimulation of the afferent nerve up to a frequency of 55 per second, and, by a mechanical resonance method, up to a frequency of 65 per second. By a "doubling frequency" method, it is shown further that the frequency-rate of the reflex discharge has not reached its limit under a stimulation of 75 per second, but surpasses that degree, though to what extent the method cannot say.

3. The maximal mechanical power of a muscle contracting under spinal reflex action is sometimes as great as the maximal which can be evoked from it by direct faradisation of the motor nerve itself.

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* M. Camis, 'Journ. Physiol.,' vol. 39, p. 228 (1909).

CROONIAN LECTURE.—*The Physiological Basis of Thirst.*

By Major WALTER B. CANNON, M.R.C., U.S. Army;
George Higginson Professor of Physiology, Harvard University.

(MS. received and Lecture delivered June 20, 1918.)

A custom which has usually been respected by investigators who in years past have had the high honour of delivering the Croonian Lecture is that of reporting and interpreting a group of related researches upon which they have been engaged and which they have already made public. That is a custom which I should have been happy to follow on the present occasion if military service had not sharply broken in on my studies months ago and made them seem now very remote and the summarising of them a difficult occupation. And, after all, is it not natural for us as investigators to hold the forward look, to consider the problems before us rather than those that have been solved? May I, therefore, be permitted to bring to your attention some ideas and observations which have not yet been published and which, though incomplete, may prove interesting and suggestive.

In regarding the human body as a self-regulating organisation we observe that, so far as mere existence is concerned, it depends on three necessary supplies from the outer world,—on food, to provide for growth and repair and to yield energy for internal activities and the maintenance of body heat; on oxygen, to serve the oxidative processes essential to life; and on water, as the medium in which occur all the chemical changes of the body. These three supplies are of different orders of urgency. Thus a man may live for 30 or 40 days without taking food, as professional fasters have demonstrated,* and suffer no apparent permanent injury to his bodily structure or functions. On the other hand, lack of oxygen for only a brief period may result in unconsciousness and death. Indeed, certain nerve cells in the cerebral cortex cannot withstand total deprivation of oxygen for more than 8 or 9 minutes without undergoing such fundamental changes that they do not again become normal when they receive their proper supply.† Intermediate between the long survival without food and the very brief survival without oxygen is the period of existence which is possible without water. Records of men who have missed their way in desert regions and who, with no water to drink, have wandered in the scorching heat have proved that they rarely live under these circumstances of struggle and torrid atmosphere for more than three days, and many die within 36 hours. An exceptional instance has been reported, of a Mexican, who, lost in the dry plains of the south-western part of the United States, walked, or crept on his hands and knees, between 100 and 150 miles, repeatedly drinking his own excretions, and succeeded, after nearly 7 days wholly without water, in reaching a habitation.* This is a record which, for its conditions, has no parallel. If the thirsting man is not subjected to heat or exertion his life may continue much longer than 7 days. Viterbi, an Italian political prisoner, who committed suicide by refusing food and drink, died on the eighteenth day of his voluntary privation. After the third day the pangs of hunger ceased, but, until almost the last, thirst was always more insistent and tormenting. He records again and again his parched mouth and throat, his burning thirst, his ardent and continual thirst, his thirst constant and ever more intolerable.† Thus though the period of survival varies, death is sure to come whether food, or oxygen, or water is withheld.

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* Luciani, 'Das Hungern,' Leipzig, 1890.

† See Gomez and Pike, 'Jour. of Exp. Med.,' vol. 11, p. 262 (1909).

Normally these three supplies—food, oxygen, and water—are maintained in more or less constant adjustment to the bodily needs. Food material is being continually utilised in building body structure, and in providing energy for bodily activities, but it is periodically restored. Oxygen is continually combining with carbon and hydrogen and leaving the body in CO_2 and H_2O, but the loss is compensated for with every breath. And water, likewise, is always being discharged in expired air, in secretion from the kidneys, and in the sweat. So great is the escape by way of the lungs and skin alone that it is estimated that approximately 25 per cent. of the heat loss from the body is due to evaporation from these surfaces.‡ This continuous lessening of the water content must be checked by a new supply, or important functions will begin to show signs of need.

The evidence for the absolute necessity of water in our physiological processes requires no elaboration. Water is a universal and essential ingredient of all forms of organisms. Without it life disappears or is latent—the dry seed awakens only on becoming moist. Because we may have it at almost any moment we are likely to overlook its absolute necessity in our lives. Among inhabitants of desert regions, however, water is the central nucleus of thought about which all other ideas revolve; it is an ultimate standard of things, incomparably more stable and more exalted than the gold of civilised commerce, the constantly remembered basis of existence.§ In our bodies the presence of water as the main constituent of the digestive secretions, its *rôle* in the chemical changes of digestion, its service as a vehicle of absorption, its importance in the composition of blood and lymph, its use, together with other substances, in body fluids as a lubricant, its action in regulating body temperature—these functions need merely to be mentioned to illustrate how water influences every activity which living beings display.

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* McGee, 'Interstate Med. Jour.,' vol. 13, p. 279 (1906).

† Viterbi, quoted by Bardier, Richet's 'Dictionnaire de Physiologie,' article "Faim," vol. 6, p. 7 (1904).

‡ Gephart and Du Bois, 'Arch. Int. Med.,' vol. 17, p. 902 (1916).

§ McGee, "The Seri Indians," '17th Annual Report of the Bureau of American Ethnology,' p. 181.

Because water is a fundamental essential to life, and is continually escaping from the body, and because there is consequent need for repeated replenishment of the store, an inquiry into the mechanism of the replenishment is a matter of interest.

That such a mechanism exists is indicated by the fact that all our essential functions, leading to preservation of the individual and of the race, are controlled not through memory and volition, but by insistent sensations and desires. The unpleasant sensation of thirst causes us to drink. Not towards the subjective aspect of these automatic arrangements, however, is the special attention of the physiologist directed. He is primarily concerned with the bodily states which give rise to the sensation. Only when these states and their relations to the needs of the organism are known is the automatic control explained.

About six years ago I called attention to some graphic records of motions of the stomach in man which showed that the sensation of hunger is associated with powerful contractions of the empty or nearly empty organ. And because the hunger pang began to be experienced after the contraction had started, the conclusion was drawn that hunger is not a "general sensation," as was formerly held by physiologists and psychologists, but has its immediate origin in the stomach, and is the direct consequence of the strong contraction.* This conclusion has since been abundantly confirmed by Carlson and his collaborators in observations on themselves and on a man with a gastric fistula.†

Even more imperious than hunger as an insistent and tormenting sensation, accompanied by a dominant impulse which determines our behaviour, is thirst. Indeed, these two experiences—hunger and thirst—are such impelling motives in directing our conduct that from early times they have been used as supreme examples of a strong desire. The ancient prophet spoke of a "hunger and thirst after righteousness" to express the eagerness of his yearning. And the common acquaintance of mankind with the potent demands of hunger and thirst for satisfaction renders these similes easily understood.

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* The Harvey Lectures, New York, 1911–1912, p. 130—Cannon: Harvey Lecture, December 16, 1911, "A Consideration of the Nature of Hunger"; also Cannon and Washburn, 'Am. Jour. Physiol.,' vol. 29, p. 441 (1912).

† Carlson, 'Control of Hunger in Health and Disease,' Chicago, 1916.

In undertaking a discussion of thirst it is necessary at the start to distinguish clearly between the primitive sensation itself and appetite. The same distinction had to be drawn in considering the nature of hunger. The hunger pang is a disagreeable ache or gnawing pain referred to the lower mid-chest region or the epigastrium. Appetite for food, on the other hand, is related to previous experiences which have yielded pleasurable sensations of taste or smell. Thus associations become established between particular edible substances and the delights they convey, with the result that a wish develops that the delights may be renewed. In either circumstance, whether for the satisfaction of appetite or for the satisfaction of hunger, the body is supplied with food.

Similarly in the case of drink, the appetite for this or that peculiar potable substance develops from former experience and from established associations of an agreeable character. We drink not only because we are thirsty, but also because we relish a certain aroma or bouquet, or a peculiar taste, and wish to enjoy it again. In respect to appetite the taking of fluid differs from the taking of food, in that fluid, which leaves the stomach rapidly, may not occasion a sense of satiety as does food, which accumulates in the stomach. In this possibility of continuing pleasurable sensations associated with drinking lie the dangers that arise from the excessive use of beverages. Under normal conditions, however, it is through the satisfaction of appetite for a particular drink, *e.g.*, for tea, or coffee, or light alcoholic beverages, that the body may be supplied with sufficient water for its needs before thirst has had occasion to manifest itself. But just as there is provided, back of the appetite for food, in readiness to become imperious if necessary, the sensation of hunger; so likewise, as a final defence against a too great depletion of the water content of the body, there may appear the urgent and distressing sensation of thirst.

There is a general agreement that thirst is a sensation referred to the mucous lining of the mouth and pharynx, and especially to the root of the tongue and to the palate. McGee, an American geologist of large experience in desert regions, who made numerous observations on sufferers from extreme thirst, has distinguished five stages through which men pass on their way to death from lack of water.* In the first stage there is a feeling of dryness in the mouth and throat, accompanied by a craving for liquid. This is the common experience of normal thirst. The condition may be alleviated, as everyday practice demonstrates, by a moderate quantity of water, or through exciting a flow of saliva by taking into the mouth fruit acids such as lemon or tomato juice, or by chewing insoluble substances. In the second stage the saliva and mucus in the mouth and throat become scant and sticky. There is a feeling of dry deadness of the mucous membranes. The inbreathed air feels hot. The tongue clings to the teeth or cleaves to the roof of the mouth. A lump seems to rise in the throat, and starts endless swallowing motions to dislodge it. Water and wetness are then exalted as the end of all excellence. Even in this stage the distress can be alleviated by repeatedly sipping and sniffing a few drops of water at a time. "Many prospectors," McGee states, "become artists in mouth moistening, and carry canteens only for this purpose, depending on draughts in camp to supply the general needs of the system." The last three stages described by McGee, in which the eyelids stiffen over eyeballs set in a sightless stare, the distal tongue hardens to a dull weight, and the wretched victim has illusions of lakes and running streams, are too pathological for our present interest.

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* McGee, 'Interstate Med. Jour.,' vol. 13, p. 279 (1906).

The fact I wish to emphasise is the persistent dryness of the mouth and throat in thirst. Direct testimony is given by King, a medical officer in a United States Cavalry troop, which for 3½ days was lost without water in the torrid "Llano Estacado" of Texas. He records that, on the third day, salivary and mucous secretions had long been absent, and that mouths and throats were so parched that food, on being chewed, gathered about the teeth and in the palate, and could not be swallowed. "Sugar would not dissolve in the mouth."*

Further evidence of the relation between local dryness of the mouth and throat and the sensation of thirst is found in some of the conditions which bring on the sensation. Breathing hot air free from moisture, prolonged speaking or singing, the repeated chewing of desiccated food, the inhibitory influence of fear and anxiety on salivary secretion, have all been observed to result in dryness of the buccal and pharyngeal mucous membrane and in attendant thirst. On the other hand, conditions arising in regions remote from the mouth and involving a reduction of the general fluid content of the body, such as profuse sweating, the excessive diarrhœa of cholera, the diuresis of diabetes, as well as such losses as occur in hæmorrhage and lactation, are well recognised causes of the same sensation. There appear to be, therefore, both local and general origins of thirst. In correspondence with these observations, two groups of theories have arisen, just as in the case of hunger—one explaining thirst as a local sensation, the other explaining it as a general and diffuse sensation. These theories require examination.

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* King, 'Amer. Jour. Med. Sci.,' vol. 75, p. 404 (1878).

The view that thirst is a sensation of local origin has had few advocates, and the evidence in its favour is meagre. In 1885 Lepidi-Chioti and Fubini* reported observations on a boy of 17, who, suffering from polyuria, passed from 13 to 15 litres of urine daily. When prevented from drinking for several hours, this youth was tormented by a most distressing thirst, which he referred to the back of the mouth, and at times to the epigastrium. The observers tried the effect of brushing the back of the mouth with a weak solution of cocaine. Scarcely was the application completed before the troublesome sensation wholly ceased, and the patient remained comfortable from 15 to 35 minutes. If, instead of cocaine, water was used to brush over the mucous membranes, thirst was relieved for only two minutes. The temporary abolition of a persistent thirst by use of a local anæsthetic, in a human being who could testify regarding his experience, is suggestive support for the local origin of the sensation. The evidence adduced by Valenti is also suggestive. He cocainised the back of the mouth and the upper œsophagus of dogs which had been deprived of water for several days, and noted that they then refused to drink.† One might suppose that the refusal to take water was due to inhibition of the swallowing reflex by anæsthetisation of the pharyngeal mucosa, as reported by Wassilief.‡ But Valenti states that his animals are quite capable of swallowing.§

Though these observations are indicative of a local source of the thirst sensation, they leave unexplained the manner in which the sensation arises. Valenti has put forward the idea that all the afferent nerves of the upper part of the digestive tube are excitable to stimuli of thirst, but that suggestion does not advance our knowledge so long as we are left unenlightened as to what these stimuli are. A similar criticism may be offered to Luciani's theory that the sensory nerves of the buccal and pharyngeal mucosa are especially sensitive to a diminution of the water-content of the circulating fluid of the body; indeed, that these nerves are advance sentinels, like the skin nerves for pain, warning the body of danger.‖ No special features of the nerves of this region, however, are known. No special end-organs are known. The intimation that these nerves are peculiarly related to a general bodily need is pure hypothesis. That they mediate the sensation of thirst is unquestioned. But the problem again is presented, How are they stimulated?

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* Lepidi-Chioti and Fubini, 'Giorn. d. R. Accad. d. Med.,' Turin, vol. 48, p. 905 (1885).

† Valenti, 'Arch. Ital. de Biol.,' vol. 53, p. 94 (1910).

‡ Wassilief, 'Ztschr. f. Biol.,' vol. 24, p. 40 (1888).

§ Valenti, 'Cbl. f. Physiol.,' vol. 20, p. 450 (1906).

‖ Luciani, 'Arch. di Fisiol.,' vol. 3, p. 541 (1906).

The view that thirst is a general sensation was well stated by Schiff. It arises, he declared, from a lessened water-content of the body, a condition from which the whole body suffers. The local reference to the pharynx, like the local reference of hunger to the stomach, is due to association of experiences. Thus the feeling of dryness in the throat, though it accompanies thirst, has only the value of a secondary phenomenon, and bears no deeper relation to the general sensation than heaviness of the eyelids bears to the general sensation of sleepiness.* The conception of thirst, as a general sensation, is commonly accepted, and is supported by considerable experimental evidence. The interpretation of this evidence, however, is open to question, and should be examined critically.

First among the experiments cited are those of Dupuytren and the later similar experiments of Orfila.† These observers abolished thirst in dogs by injecting water and other liquids into the dogs' veins. And Schiff quotes Magendie as having treated successfully by the same procedure the thirst of a patient suffering from hydrophobia. In these instances the treatment was no doubt general, in that it affected the body as a whole. But the assumption that thirst is thus proved to be a general sensation is unwarranted, for the injection of fluid into the circulation may have changed local conditions in the mouth and pharynx, so that the local sensation no longer arose.

A classic experiment repeatedly cited in the literature of thirst was one performed by Claude Bernard. He opened a gastric fistula which he had made in a dog, and allowed the water which the animal drank to pass out. As the animal became thirsty, it would drink until "fatigued," as the report states, and when "rested" it would begin again. But after the fistula was closed, drinking quickly assuaged the desire for water. The inference was drawn that thirst must be a general sensation, for the passage of water through the mouth and pharynx wet those surfaces, and yet the animal was not satisfied until the water was permitted to enter the intestine and be absorbed by the body.‡ This evidence appears conclusive. The expressions "fatigued" and "rested," however, are interpretations of the observer, and not the testimony of the dog. Indeed, we may with equal reasonableness assume that the animal stopped drinking because he was not thirsty, and started again when he became thirsty. The only assumptions necessary for such an interpretation of the animal's behaviour are that appreciable time is required to moisten the buccal and pharyngeal mucosa sufficiently to extinguish thirst—a point made by Voit*—and that these regions become dried rapidly when there is absence of an adequate water-content in the body. This interpretation is consistent with the view that thirst is a sensation having a local source. Furthermore, this interpretation is not contradicted by the satisfaction manifested by the dog after the fistula was closed, for the water which is absorbed, like that injected into veins, may quench thirst by altering local conditions. We cannot admit, therefore, that Bernard's experiment is proof that thirst is a general sensation.

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* Schiff, 'Physiologie de la Digestion,' Florence and Turin, vol. 1, p. 41 (1867).

† See 'Dictionnaire des Sci. Méd.,' Paris, vol. 61, p. 469 (1821).

‡ Bernard, 'Physiologie Expérimentale,' Paris, vol. 2, p. 49 (1856).

Another set of observations cited as favourable to the theory of the diffused character of the origin of thirst are those of Longet. After severing the glosso-pharyngeal, the lingual and the vagus nerves on both sides in dogs, he observed that they drank as usual after eating.† If thirst has a local origin in the mouth and pharynx, why should the animals in which the nerves to these regions were cut still take water? Two answers to this question may be given. First, as Voit has pointed out,‡ Longet did not cut all branches of the vagi and trigemini to the mouth and pharynx, and, consequently, some sensation persisted. And second, even if all nerves were cut, the fact that the animals drank would not prove that thirst exists as a general feeling, for one may drink from the sight of fluid, or from custom, without the stimulation of a dry mouth, just as one may eat from the sight of food without the stimulus of hunger. In other words, the element of appetite, previously considered, may enter, and as a matter of habit and associated experience determine present reactions.

The remaining evidence in favour of the diffused origin of thirst is found in studies of blood changes. These changes, by altering the "milieu intérieur" of the body cells, must affect them all. In 1900, Mayer published reports on the increase of osmotic pressure of the blood, as determined by depression of the freezing point of the serum, which he noted in conditions naturally accompanied by thirst. Dogs deprived of water for several days had a blood serum in which the osmotic pressure was increased, and rabbits kept in a specially warmed chamber showed the same change. Thus, conditions in which the water supply to the body was stopped, or the loss of water from the body by sweating or pulmonary evaporation was increased, either of which is known to cause thirst, were associated with a rise of osmotic pressure. And Mayer argued that all other circumstances in which thirst appears—in diabetes with increased blood sugar, in renal disease with accumulation of waste material in the body fluids, in acute rabies with total deprivation of water, in cholera with excessive outpouring of water into the intestine—the osmotic pressure of the blood would be augmented. Moreover, when a thirsty dog drinks, the hypertonicity of his serum disappears, his normal condition is restored, and he stops drinking.

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* Voit, 'Hermann's Handbuch der Physiologie,' Leipzig, Abth. 6, p. 566 (1881).

† Longet, 'Traité de Physiologie,' Paris, vol. 1, p. 35 *et seq.* (1868).

‡ Voit, *loc. cit.*

By these observations Mayer was led to the conclusion that whenever the osmotic pressure of the blood rises above normal, thirst appears; whenever it returns to normal, thirst vanishes; and as the pressure varies, thirst also varies. Since intravenous injections of hypertonic salt solution cause, by stimulation of the bulbar centres, according to Mayer, a rise of arterial pressure and renal and intestinal vasodilation—both operating to lower the abnormally high osmotic pressure of the blood—he infers that other agencies are present in the organism besides the desire for water, which tend to keep the blood normal. Thirst, he declares, is the last of a series of mechanisms acting to protect the organism against hypertonicity of its fluids.

In summary, then, the thirsty individual has a blood with high osmotic pressure. This condition affects all the cells of the body. It disturbs the cells of the central nervous system, and thus leads both to protective circulatory reactions and, in case these fail, to malaise and irritability, and a reference of unpleasantness to the region of the pharynx. Accompanying this, there is the impulse to drink, and when that is satisfied, the water taken in restores the normal state.*

Mayer's observations were soon confirmed, but his inferences were challenged. In 1901, Wettendorff, working in Brussels, reported that if dogs are deprived of water their blood does, indeed, develop a hypertonicity, as Mayer had found, but that this is a phenomenon which does not occur to any marked degree in the first days of the deprivation. In one instance there was no change in the freezing point of the serum during three days of thirst. Serious alteration of the osmotic pressure of the blood, therefore, is comparatively tardy in its appearance. Since the organism is continually losing water, and, nevertheless, the blood remains for a day or two unchanged, Wettendorff concluded that the consistency of the blood is preserved as long as possible by withdrawal of water from the extravascular fluids and the tissues. Further, thirst is clearly demonstrable long before any considerable change in the blood is evident. One animal in which the freezing point of the serum had been lowered only 0·01° C. by four days' deprivation of water, drank 200 c.c. of physiological salt solution, a liquid which to the dog in normal condition is quite repugnant. Again, when the blood has become slightly hypertonic, a dog may drink normal salt solution without lowering his osmotic pressure and afterwards, by refusing further drink, act quite as if he had slaked his thirst. But if an animal with a very hypertonic blood is placed before hypertonic salt solution he takes it again and again—an action which may be explained by a draining of water from the tissues with increasing intensity, and a consequent increasing thirst.

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* Mayer, 'C. R. Soc. de Biol.,' vol. 52, pp. 154, 389, 522 (1900); also 'Essai sur la Soif,' from the Laboratory of Experimental Pathology, Faculty of Medicine, Paris, 1900.

From all these observations Wettendorff concluded that the origin of thirst does not reside in alterations of the blood itself, but in the act of withdrawing water from the tissues. The liquids bathing the cells, therefore, would be first to concentrate as water is lost from the organism. And since the conditions of cellular life would thus be modified in all the tissues, the peculiar state would develop which occasions the sensation of thirst. This effect is generally diffused, and is independent of any peculiar influence of the process of dehydration on the nervous system itself.

In accounting for the localising of the sensation in the mouth and throat Wettendorff distinguished between a "true thirst" and a "false thirst." "True thirst," he declared, is dependent on an actual bodily need, and is persistent until the need is satisfied. "False thirst" is only a dryness of the mouth and pharynx. Dryness in this region occurs, to be sure, in true thirst, but it is then an expression of the general dehydration of the tissues, exaggerated perhaps by contact with the outer air. Through experience the two conditions—buccal dryness and general dehydration—have become associated. Even in true thirst we may temporarily abolish the sensation by moistening the pharyngeal mucous membrane, but the result is only a "false satisfaction," a self-deception, made possible because long and pleasant experience has proved that moistening this region by drink leads to the satisfaction of an instinctive need.*

The foregoing review of observations and theories has revealed that the attitude of physiologists with reference to thirst has been much as it was with reference to hunger. In each condition a general bodily need has arisen from a lack of essential bodily material and is signalled by a well-defined sensation. In each the testimony of ingenuous persons regarding their feelings has been carefully set down, and then explained away. Thus in the case of thirst the primary sensation is described universally as an experience of dryness and stickiness in the mouth and throat.† Instead of attempting to account for the experience as such, however, attention has been paid to the bodily need which accompanies it; apparently, since the need is a general one, the sensation has been supposed to be general, and the thirst which everybody experiences and knows about has been classed as an associated secondary phenomenon or the peripheral reference of a central change. The really doubtful feature in this view of thirst, just as in the older conception of hunger, is the "general sensation." That even the early stages of a need of water may be accompanied by increased irritability, and a vague sense of weakness and limpness, is not denied. But the thirsty man does not complain of these general conditions. He is tormented by a parched and burning throat, and any explanation of the physiological mechanism for maintaining the water content of the body must take into account this prominent fact.

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* Wettendorf, 'Travaux du Laboratoire de l'Institut Solvay,' Brussels, vol. 4, pp. 353–484 (1901).

† Foster, 'Textbook of Physiology,' London, p. 1423 (1891); Ludwig, 'Lehrbuch der Physiologie,' vol. 2, p. 586; Voit, 'Hermann's Handbuch der Physiologie,' Abth. 6, p. 566.

In looking for a mechanism which would automatically keep up the water supply of our bodily economy, we may follow two clues; first, that there may be a peripheral arrangement which in the presence of a general bodily need for water would lead to dryness of the mouth and throat; and second, that a peripheral arrangement of this nature should be especially characteristic of animals which are constantly and rapidly losing water and require repeated renewal of the supply. These two clues offer a biological approach to the explanation of thirst which I wish to utilise.

In one sense all animals are constantly losing water, for even in the simplest forms waste material is excreted in solution. With respect to water loss, however, we should expect to find a marked difference between animals living in water itself and those living in air. Indeed, it is difficult to conceive of an animal living in water as experiencing thirst. The entire body surface and the mouth and throat are throughout active life continuously bathed in a moving flow. The food is taken wet from a wet medium. Probably renal activity and the secretion of the digestive glands are the only important ways for water to leave the economy; and the digestive secretions are soon largely re-absorbed. In contrast, the land animals, mammals, for example, lose moisture not only in these ways but also by the moistening of dry food, by evaporation from the extensive surface of the lungs, and by the action of innumerable sweat glands. It is because of the possibility of great and rapid loss of water from its body that the land animal has special need for an assurance of adequate supply.

In the water inhabitant the skin, and the mouth and gullet, are all kept wet by the medium in which he lives and moves. In the process of evolution, however, as organisms changed their habitat from water to air, the skin became dry and scaly. Of the parts which in marine animals were constantly bathed by water, only the mouth and throat continue to be moist. These regions are now exposed to air, however, instead of being flushed by a flowing stream, and consequently they tend to dry. The structural lining of these parts probably renders them especially liable to desiccation in the presence of dry air, for the mucosa of the mouth and also of the pharynx, below the level of the floor of the nasal chambers, is composed of squamous epithelium Some scattered mucous glands are present, but they are not capable of keeping the surfaces satisfactorily wet, as any one can readily prove by breathing through the mouth for only a few minutes. When air passes to and fro by way of this watercourse, as in prolonged speaking or singing, and in smoking, it is to be expected, therefore, that feelings of dryness and stickiness, which we call thirst, should arise.

Contrast this condition of the mouth with the condition of the respiratory tract, in which the lining membrane consists of columnar epithelium and is richly provided, particularly in the nose, with multitudes of mucous glands. Through this tract air moves to and fro constantly with no sign of inducing desiccation except in extreme and prolonged deprivation of water. But there is one portion of this normal pathway for the air which, in the absence of sufficient moisture, is peculiarly liable to become dried. It is the pharynx, where the respiratory tract crosses the digestive tract—*i.e.*, where the inbreathed air, which may be insufficiently moistened in the nose, passes over surfaces of the ancient watercourse. Here, even with nasal respiration, unpleasant feelings may be excited, if the water-content of the body is reduced, and, in cases of marked thirst, the dryness of this region may stimulate tireless swallowing motions.

The central questions now appear: Why do not the mouth and pharynx feel dry and uncomfortable under normal conditions? and why do they feel so when the body stands in need of water? Again, a comparison of conditions in the water inhabitants, in which the buccal and pharyngeal regions are kept moistened by the surrounding medium, with conditions in the air inhabitants, in which these regions tend to be dried by the surrounding medium, will offer pertinent suggestions. A characteristic difference between these two animal groups is the possession, by the air inhabitants, of special buccal glands. They are not present in fishes, but are found in the rest of the vertebrate series from the amphibia onwards. At first little differentiated, they develop in mammals into the three pairs of salivary glands—the parotid, sub-maxillary, and sub-lingual. For the purpose of considering thirst in man, we may deal solely with this salivary group. The action of these organs is to secrete a fluid which is normally more than 97 per cent., and may be more than 99 per cent., water.* The theory of thirst, on which I wish to offer evidence, may now be stated. In brief, it is that the salivary glands have, among their functions, that of keeping moist the ancient watercourse: that they, like other tissues, suffer when water is lacking in the body—a lack especially important for them, however, because their secretion is almost wholly water, and that, when these glands fail to provide sufficient fluid to moisten the mouth and throat, the local discomfort and unpleasantness which result constitute the feeling of thirst.

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* Becker and Ludwig, 'Ztschr. f. Rat. Med.,' vol. 1, p. 278 (1851).

That one of the uses of buccal glands is to keep wet the surfaces over which their secretion is distributed is indicated by the fact that these structures first appear in air-inhabiting vertebrates. This indication receives support from the conditions seen in the cetacea, the mammalian forms which have returned to an aquatic existence, and in which both the water-loss from the body and the need for wetting the mouth and throat are greatly reduced. It is a remarkable fact that in these animals the salivary glands are either lacking or are very rudimentary. The appearance and disappearance of the buccal glands in large animal groups, in correspondence with the exposure or non-exposure of the mouth and throat to desiccating air, point to these glands as protectors of the buccal mucosa against drying.

Experimental evidence as to the protective function of the salivary secretions was provided incidentally many years ago by Bidder and Schmidt. They were interested in studying any fluid secretion which might appear in the mouth apart from saliva. To this end they tied in dogs all the salivary ducts. The first effect was such a striking diminution of the fluid layer over the buccal mucosa that only when the mouth was held closed was the surface kept moist, and, when the animal breathed through the mouth, a real drying of the surface was hardly prevented. The eagerness for water, they state, was enormously increased, so that the animal was always ready to drink.*

Related to this service of saliva in moistening and lubricating the mouth parts is the presence of a special reflex for salivary secretion when the buccal mucosa is exposed to conditions which tend to dry it. Thus, as Pavlov's† researches demonstrated, with dry food in the mouth, much more saliva is secreted than with moist food. And Zebrowski‡ found, in the course of observations on patients with a parotid fistula, that, whereas no saliva flowed with the mouth closed, as much as 0·25 c.c. in five minutes came from the duct when the mouth was opened. This reflex is readily demonstrated. If one closes the nostrils and breathes through the mouth for five minutes, usually nothing happens during the first minute. The mucosa then begins to feel dry, and at once the saliva starts flowing, and continues for the rest of the period. I have thus collected as much as 4·7 c.c. in four minutes. Chewing motions, with the mouth empty, yielded in five minutes only about 1 c.c. In these observations precautions were taken against any psychic effect due to interest, by adding long columns of figures during the test. It seems clear, therefore, that if the mouth tends to become dry, the salivary glands are normally stimulated to action, and, if there is sufficient outflow from them, the affected surfaces are moistened. The act of swallowing favours the process, for the fluid is thereby spread backwards on the tongue and wiped down the back wall of the pharynx.

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* Bidder and Schmidt, 'Verdauungssäfte und Stoffwechsel,' Leipzig, p. 3 (1852).

† Pavlov, 'The Work of the Digestive Glands,' London, 2nd ed., pp. 70, 82 (1910).

‡ Zebrowski, 'Arch. f. d. ges. Physiol.,' vol. 110, p. 105 (1905).

The question whether there is a relation between the existence of water-need in the body and diminished flow of saliva I have examined in two ways —by going without fluid for a considerable period and by profuse sweating, combined with measurements of salivary secretion under uniform stimulation. The method of determining salivary output was that of chewing for five minutes and at a uniform rate a tasteless gum, collecting the saliva which flowed during this period, and measuring its volume. All these observations are best made when one is inactive, and in my experience more nearly uniform results are obtained if one lies quiet during the tests.

The influence on salivary flow of going without fluid for some time may be illustrated by an example. The chewing to evoke salivary action was started at 7 o'clock in the morning, and repeated each hour until 8 o'clock in the evening. A breakfast consisting of a dry cereal preparation was taken between 8 and 9 o'clock, and a luncheon of dry bread between 12 and 1 o'clock. Nothing had been drunk since the previous evening. From the first test at 7 o'clock until 11 there was little change in the output of saliva; the average amount secreted in 5 minutes was 14·1 c.c., with variations between 13 and 16·4 c.c. Then the output began to fall, and at 2 o'clock only 6·4 c.c. was secreted. The average amount for the two observations at 2 and 3 o'clock was 7·7 c.c.—only little more than half that poured out in the morning. Between 3 and 4 o'clock a litre of water was drunk. The effect was soon apparent. At 4 o'clock the output was 15·6 c.c., and during the next 4 hours, in which more water was taken, and a supper with thin soup and other fluid was consumed, the average amount secreted was 14·6 c.c., a figure closely corresponding to the 14·1 c.c. of the morning hours. These results are illustrated graphically in fig. 1. Other tests of this character gave similar results, though there was variation in the rate of decrease in the amounts of saliva secreted.

A similar diminution of the salivary secretion occurs after the loss of water from the body by sweating. In one instance, the loss in about one hour of approximately 500 c.c. of body fluid as sweat was accompanied by a reduction in the salivary output of almost 50 per cent.

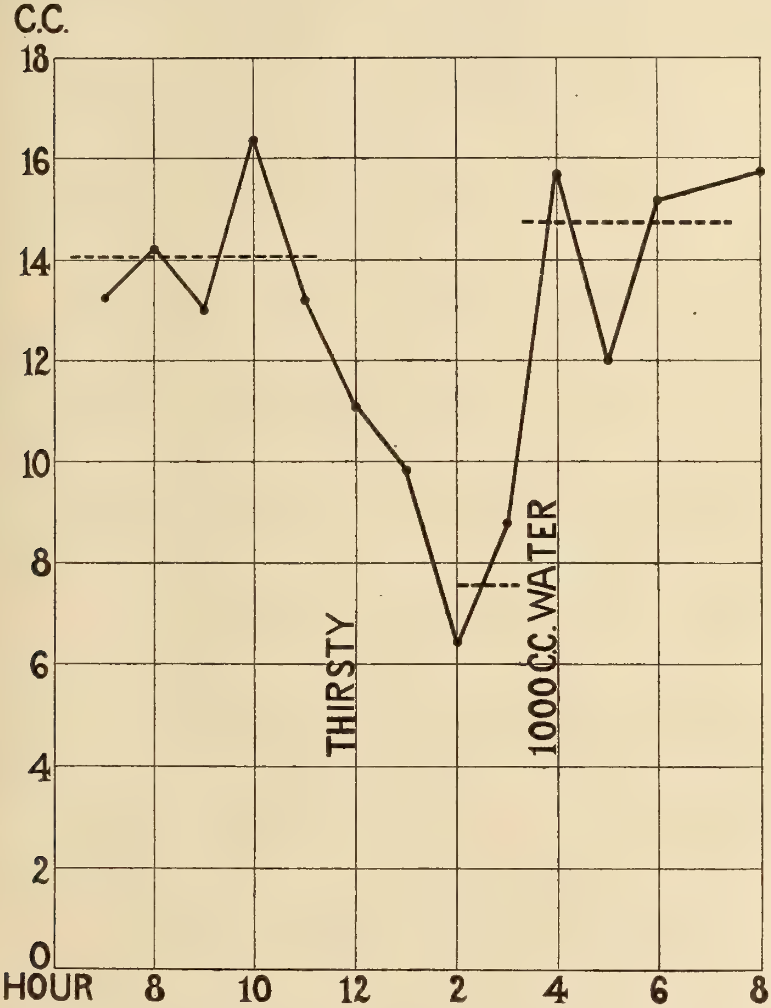

Fig. 1.—Chart showing saliva secreted each hour from 7 A.M. until 8 P.M. in consequence of chewing a tasteless gum five minutes. No fluid was drunk between 7 o'clock the previous evening and 3 o'clock P.M. For further description, see text.

Corresponding to the diminution of the salivary output as the result of chewing was a diminution in the reflex flow as a consequence of letting the mouth become dry. The reflex flow has fallen, in my experience, from 3 or 4 c.c. in five minutes under normal conditions to a little more than 1 c.c. during thirst.

The relation between the decrease of salivary flow in these experiments and the sensation of thirst was quite definite. In the experiment illustrated in fig. 1, for example, the feeling of being "thirsty" was absent until the secretion of saliva began to decline, after 11 o'clock. From that time onward the back of the throat began to feel dry; there was frequent swallowing, and both the movements of the tongue and the act of deglutition were associated with a sense of "stickiness," a lack of adequate lubrication of the parts. All of this unpleasantness and discomfort disappeared after the restoration of the saliva flow by drinking water.

The increased spontaneous activity of the tongue and the repeated swallowing motions as "thirst" became more marked are noteworthy. These movements are a slight stimulus to salivary secretion, and they have, furthermore, the obvious effect of spreading about any fluid that might be present. In the absence of sufficient fluid, however, they augment the disagreeableness of the condition by making prominent the friction due to lack of lubricant. The "lump in the throat," which is complained of by persons who suffer from extreme thirst, can be explained as due to the difficulty encountered when the epiglottis and root of the tongue are rubbed over the dry back wall of the pharynx in attempts to swallow.

The only statement that I am aware of, which is contradictory to the evidence just presented, is that made by one of a group of psychologists, reported by Boring.* This one observer testified that when he was beginning to be thirsty the saliva flow was still copious. The eight other observers of the group speak of thirst as being characterised by dryness of the roof of the mouth, dryness of the lips, the sensation of having a "dry sore throat," feelings of stickiness, and uncomfortable "puckery" pressure localised in the middle and back of the tongue and in the palate—in other words, as one of them summed up his experience, "dryness expresses the complex as a whole." This body of testimony agrees closely with that presented earlier and suggests that there may have been error in the one observation that thirst was associated with free secretion of saliva.

Other evidence on the relation between absence of saliva and the presence of thirst as a sensation was obtained through checking salivary secretion by atropine. Before the injection the amount secreted during 5 minutes by chewing averaged 13·5 c.c. After the full effect of the drug was manifest, the amount fell to 1 c.c. All the feelings that were noted in ordinary thirst—the sense of dry surfaces, the stickiness of the moving parts, the difficulties of speaking and swallowing—all were present. These disagreeable experiences, constituting the thirst sensation, disappeared as soon as the mouth and throat were washed out with a weak novocaine solution. The immediate effect in these circumstances was doubtless due to the water in the solution, but since the relief lasted much longer than when water was used, the anæsthetic was also a factor. This experience agrees with that of Lipidi-Chioti and Fubini, mentioned earlier. No water was drunk by me during the period of atropine effect, and yet when that effect disappeared, and the saliva flow was re-established, thirst also was abolished. The relation between thirst and such drug action has been noted before, but so strong has been the theory that thirst is a "general" sensation, that the drug has been supposed to produce its effect not by local action but by central changes and by alteration of the blood.*

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* Boring, 'The Psychological Review,' vol. 22, p. 307 (1915).

Similar in character to the thirst which results from the action of atropine is that which accompanies anxiety and fright. The effect of such emotional states in causing inhibition of salivary secretion is well known. It was the basis of the ancient "ordeal of rice" employed in India as a means of detecting the guilty one in a group of suspected persons. It is illustrated in these days by Hoche's report of the effects of air raids on the people of Freiburg-in-Baden, in whom the signs of great fear—chattering of the teeth, pallor, and diarrhœa—were attended by intense thirst.† The unquenchable nature of the thirst which results from terror is a large part of the torment suffered by the novice in public speaking.

On the basis of the foregoing evidence I would explain thirst as due directly to what it seems to be due to—a relative drying of the mucosa of the mouth and pharynx. This may result either from excessive use of this passage for breathing, as in prolonged speaking or singing, or it may result from deficient salivary secretion. In the latter case "true thirst" exists, but it is not to be distinguished, so far as sensation is concerned, from "false thirst." True thirst is dependent on the fact that the salivary glands, which keep the buccal and pharyngeal mucosa moist, require water for their action. According to the observations and inferences of Wettendorff, the osmotic pressure of the blood is maintained, in spite of deprivation of water, by the withdrawal of water from the tissues. The salivary glands are included under "tissues," and they appear to suffer in a way which would support Wettendorff's view, for in the presence of a general need for water in the body, they fail to maintain the normal amount and quality‡ of secretion. The same is doubtless true of other glands. The importance of this failure of action of the salivary glands, however, to the mechanism of the water supply of the body, lies in the strategic position of these glands in relation to a surface which tends to become dry by the passage of air over it. If this surface is not kept moist, discomfort arises and with it an impulse to seek well tried means of relief. Thus the diminishing activity of the salivary glands becomes a delicate indicator of the bodily demand for fluid.

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* See Sherrington, 'Schäfer's Textbook of Physiology,' London, vol. 2 p. 991 (1900).

† Hoche, 'Med. Klinik,' vol. 13, p. 906 (1917).

‡ There is evidence that, as the quantity of saliva diminishes, its water content is less; *i.e.*, it is more viscous. (See Tezner, 'Arch. Intern. de Physiol.,' vol. 2, p. 153.)

The foregoing explanation is in agreement with the suggestions which have been offered to account for thirst as having a local origin. But it does not require specialised nerves, or peculiar sensitiveness of the first portion of the digestive tract, which have been assumed to be present by the upholders of this theory. And by calling attention to the arrangement by which the salivary glands are made to serve as indicators of the general bodily need for water, it presents a reasonable account of the manner in which a widespread condition of the organism may exhibit itself locally.

The experiments which have long been the chief support of the theory that thirst is a general sensation can also be explained by the evidence above adduced. The abolition of thirst by injecting fluid into the veins of thirsty animals would be expected, for, as shown in the experiment illustrated in fig. 1, by providing an adequate water supply the saliva flow is promptly re-established, and the parched mouth and throat are again continuously moistened. In the classic experiment of Claude Bernard the animal with an open gastric fistula continued to drink until the fistula was closed. This was not because there was a general demand for water throughout the body, so long as the fistula remained open, but because only when escape through the fistula was stopped did the body receive the water needed to provide the output of saliva which prevented local drying. And the dogs with salivary glands tied, described by Bidder and Schmidt, were always ready to drink, just as are persons who are terrified or who have been given atropine, because of thirst—because there is local drying of the mouth—from lack of saliva, though the body as a whole may not be in any need of water. The application of cocaine to the mucous surfaces of the mouth abolishes the torment of thirst, not by any central effect, and clearly not by satisfying any general bodily requirement for water, but by rendering the surfaces anæsthetic. The miraculous virtues of coca leaves, as a balm for the distress of the thirsty, a fact long ago observed, is explicable on these grounds. The thirst of those who suffer from loss of fluid from the body—the diabetic patient, the victim of cholera, the subject of hæmorrhage, the perspiring labourer, and the nursing mother—can be accounted for by the reduction of salivary flow as the water-content of the body is lowered, and by the consequent discomfort arising from the sticky buccal mucosa.

I am aware that many questions arising from the views which I have just developed remain to be solved—questions as to the effects which other glandular activity, removing fluid from the body, may exercise on the functions of the salivary glands; the alteration of properties of the blood and lymph other than osmotic pressure as affecting secretion; the relation between the so-called "free water" of the body fluids and salivary secretion when water is withheld; the influence of strong alcoholic beverages in producing thirst; and the nature of pathological states in which thirst seems to disappear. But these and other pertinent questions must await more peaceful times for their answers.

From the evidence presented, however, it seems to me that we are now in a position to understand the mechanisms by which all three of the essential supplies from the outer world are provided for in our bodily economy. The oxygen supply is arranged for by the control which changes in the blood, brought about mainly by variations in the carbon dioxide content, exert on the centre for respiration. The proper food supply ultimately is assured, because we avoid, or check, by taking food, the distressing pangs of hunger which powerful contractions of the empty stomach induce unless food is taken. And the water supply is maintained because we avoid, or abolish, by taking water or aqueous fluid, the disagreeable sensations which arise and torment us with increasing torment if the salivary glands, because of a lowering of the water-content of the body, lack the water they need to function, and fail therefore to pour out their watery secretion in sufficient amount and in proper quality to keep moist the mouth and pharynx.

The Mechanism and Control of Fibrillation in the Mammalian Heart.

By J. A. MacWilliam, F.R.S.

(Received June 6, 1918.)

The results of the present investigation are founded on a very extended study of the subject, carried on from time to time during the past 30 years, in the course of very numerous experiments (hundreds) on the mammalian heart.

These results establish the conclusion that in fibrillation there is an essential change in the manner of conduction of the excitation process in the cardiac musculature; the relation of this change to the excitability of the muscle determines the appearance and characters of the different forms of "fibrillar" action that may be observed. The conduction of the excitation is essentially altered, inasmuch as it is propagated along the muscular fibre systems or fasciculi, instead of travelling directly through the muscular substance, without obvious regard to the arrangement of the fibres, as in the normal beat of the heart.* Fascicular dissociation is an essential feature of fibrillation, which is, strictly speaking, a condition of "fasciculation" rather than "fibrillation." The essential change in conduction may be induced in very different ways. The state of fibrillation is rendered persistent by a disturbance in the normal relations of conduction time and refractory period in the cardiac musculature, resulting in the establishment of a mechanism of circulating excitations.

The cat's heart was the one most largely investigated, but those of rabbits, guinea-pigs, rats, etc., were also employed. The heart action was usually examined and recorded with the thorax open, while artificial respiration, by means of a pump or by continuous insufflation of the lungs with oxygen, was maintained. A myocardiograph of the type described by Cushny† was employed, arterial blood pressure or pulse being often registered at the same time. Intra-cardiac pressure records were often made from the auricles and the ventricles on the principles described by Frank. Anæsthesia was maintained by chloroform, ether, urethane, morphia, chloretone, paraldehyde, or combinations of these. In a number of experiments the method of decapitation was used. The perfused heart was frequently utilised, records being made by (*a*) the myocardiograph, used in the same way as with the heart *in situ*, and (*b*) by a rubber bag placed in the left ventricle and connected with a Hürthle manometer, the system being filled with liquid. All the tracings are to be read from left to right; they are all ventricular (L.V. of cat) records except where otherwise noted.* The time is shown in seconds.

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* See the electrocardiographic evidence advanced by Lewis and Rothschild, 'Phil. Trans.,' vol. 206, p. 181 (1915).

† 'Heart,' vol. 2, p. 1 (1910–11).

For the more accurate use of faradic currents, a Kronecker's inductorium was employed, with two volts in the primary circuit; the values of the units stated are to be taken as obtained with this E.M.F. in each case. For obtaining series of shocks at different rates, a Brodie cut-out arrangement was used, giving either make or break shocks at regular intervals; these shocks were recorded on the tracings by an electrical signal. The shocks were often applied through the myocardiograph, so that they traversed a considerable amount of the cardiac substance; at other times they were sent through electrodes about 1 mm. apart, etc.

The Conduction of the Excitation in Fibrillation.

Instead of travelling uniformly right through the mass of muscle without evident regard to the direction of the fasciculi or bands of muscle, as under normal conditions, the excitation wave in fibrillation travels most easily along the complexly-arranged fasciculi, there being an impairment or failure of propagation at most of the inter-fascicular connections. Such a mode of propagation of the rapidly-recurring contraction waves may be clearly perceived on direct inspection of the heart, and on palpation of the ventricles the apical portion being held between the finger and thumb with varying degrees of light pressure. In the latter case, instead of the normal uniform hardening of the muscular wall at systole, there is a striking want of synchronism in the hardening of the constituent fasciculi, short contraction waves in rapid succession hardening different sets of fibres, while others are relaxed and soft, the contracted ones momentarily standing out and giving a characteristic " wiry " feeling among the quiescent fasciculi; the impression of an incessant turmoil of dissociated or in-coördinated activity is a vivid one. The myocardiograph record shows a series of rapid irregular oscillations, varying to some extent from place to place in rate and in range of excursion. Similar records are obtained from the perfused heart.

The failure of normal conduction may be induced in two ways: (1) by depressing agencies acting directly on conductivity, and causing more or less extensive blocking in the most susceptible parts, the inter-fascicular junctions, while the intra-fascicular connections remain functional. This effect may be produced even with a moderate or slow succession of contractions, but is greatly favoured by rapidity of sequence of the contractions. Such depressing agencies are of various kinds—cooling, intra-vascular injection of potassium salts, bile, over-doses of many drugs, etc., including some substances that are in suitable doses useful as remedial agents promoting recovery from fibrillation; (2) by excessive rapidity of excitation, *e.g.*, by electrical stimulation. This (2) may be the sole cause of the alteration in conduction, or it may co-operate with a depressing influence acting directly on conduction, *i.e.* a combination of (1) and (2) is specially effective.

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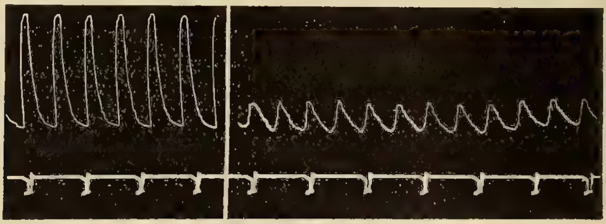
* Upward movement of the ventricular lever = systole.

Change in Mode of Conduction due to Direct Depression.

Fibrillar Beats.—That depression of conductivity is of fundamental importance is evidenced by the fact that individual beats may be "fibrillar" in character (fig. 1). This is strikingly realised on palpation; instead of the usual sensation of uniform hardening at each systole, the contraction is felt to be passing in asynchronous fashion along the different systems of fasciculi or bands of fibres, some feeling firm and contracted, with the characteristic wiry feeling, while others are soft and relaxed. On the surface of the ventricles the contraction wave is visibly slowed, and in the auricles this may be very strikingly evident in its progress over the muscle.* In this condition the nature of the ventricular beat is similar, whether it occurs in response to an impulse travelling down the A–V. conducting system, or is excited by a direct stimulus applied to the outer surface of the ventricles. The fascicular dissociation is evident even when the impulse is distributed through the endings of the Purkinje system of fibres (fig. 2).



A. B.

FIG. 1.—The systolic movement of the lever is upward. A = normal beats. B = fibrillar beats, which are strikingly wiry on palpation.

Fibrillar beats are often able to give considerable excursions of the recording lever, and they are often able to pump out a very appreciable amount of blood into the aorta. The contraction and relaxation phases are both prolonged; the systolic power is relatively small. The individual beats are quite discrete; there is a very definite interval, varying in duration, of complete quiescence between them (fig. 2). The excitability of the cardiac muscle is low when such separate beats are present; the refractory period is long. The occurrence of these fibrillar beats shows that the "fibrillar" mode of contraction is not essentially dependent on or necessarily associated with rapidity of succession at all, though the latter is a very striking feature of typical "fibrillation," giving complexity of movement, complete in-coördination, and mechanical ineffectiveness as regards expulsive power.

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* In the ventricles waves can often be plainly seen entering at or emerging from the vortex.

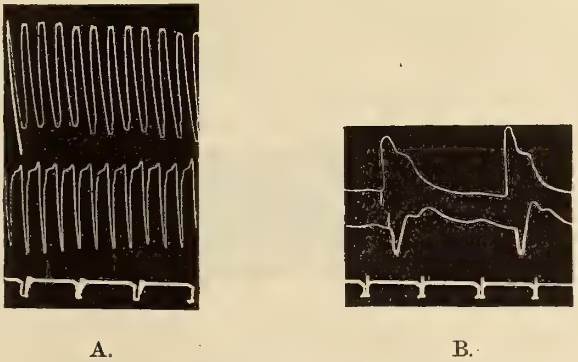


Fig. 2.—A shows normal curves, the upper one ventricular (systolic movement upward) and the lower auricular (systolic movement downward). B shows two fibrillar beats at a later phase of the experiment. Simultaneous points are marked by short vertical lines at the first beat. The Au. and V. contract together; the excitation apparently originates in the A–V. junctional tissues.

Continuous Series of Fibrillar Beats as seen in a More Excitable Heart.

When the excitability is at a higher level, or when stimulation is applied to make the fibrillar beats follow one another more quickly, a continuous succession of contraction waves appears; one fibrillar beat excites another, and they are thus strung in a series, constituting a slow coarse fibrillation (fig. 3). The rate depends on the excitability of the muscle, the degree of

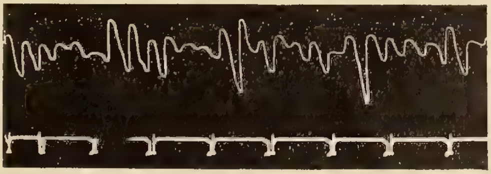


Fig. 3.—Continuous irregular series of fibrillar beats, each beat exciting a subse quent one through the mechanism of circulating excitation. An overdose (intra-vascula r) of sodium carbonate induced this condition.

dissociation varies with the rate of succession—the faster the rate the higher the grade of dissociation. In some cases the depression of conduction may be of such a degree that a beat coming after a long interval may show no distinct sign of dissociation by inspection or palpation, whereas, when a quick series occurs, each beat is markedly dissociated, giving the characteristic " wiry " feeling on palpation (fig. 4). When the excitability of such a heart gradually rises, *e.g.*, under the influence of massage, improved nutrition, certain remedial drugs, removal of depressing influences, etc., the rate of continuous movement may increase, with an accompanying increase in the grade of dissociation.

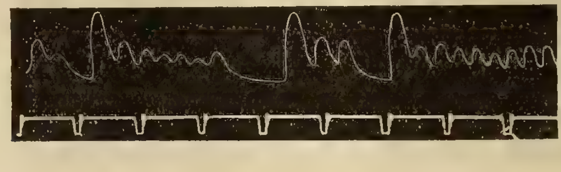


Fig. 4.—The quick series of beats are fibrillar in character. The larger beats coming after long intervals do not show evidence (on palpation) of that character.

There is a very definite gradation from (*a*) the phase of discrete fibrillar beats, through (*b*) slow and then quicker series of successive contraction waves, up to (*c*) the rapid and mechanically ineffective oscillations of typical fibrillation. The increase in rate depends on the augmented responsiveness of the more excitable muscle. The degree of asynchronism or dissociation increases with the rise in the rate of succession, the partial blocking between the larger fasciculi or bands and layers of fibres giving the lower grade of dissociation seen in slow coarse fibrillation, while the higher grades of dissociation between fasciculi are present in the condition of rapid fine fibrillation.

Similarly with diminishing excitability and conductivity, a downward gradation may be observed from typical fibrillation, through grades of slower and coarser fibrillation, to the phase of individual fibrillar beats.

Change in Mode of Conduction Due to Excessive Rapidity of Excitation.

When the rate of beat is excessively accelerated by a series of induction shocks of increasing rapidity, a gradation of changes is observable as the rate of succession rises. The individual contractions become briefer and gradually give smaller and smaller excursions of the recording lever. Inspection shows evidence of dissociation becoming very pronounced at the higher rates, so as to bear a close resemblance to the familiar appearance of the ventricular surface in typical fibrillation. Palpation at the same time reveals increasing degrees of asynchronism as the rate rises, until the characteristic wiry wriggling feeling, practically indistinguishable from that of true fibrillation, becomes very marked, instead of the solid push normally given to the palpating finger. These phenomena are obviously due to the rapid series of short contraction waves traversing, at relatively slowed rates, the various layers, bands or fasciculi of the ventricular musculature according to the lower or higher grades of inter-fascicular blocking and dissociation that are present, thus giving asynchronous contractions at different parts of the thickness of the muscular walls. These changes in their various grades are attended by related degrees of lowering of the arterial pressure, and by auricular acceleration and irregularity. At high rates the force and range of the contractions become small, the output from the ventricles is cut down and a great fall of arterial pressure results.

When the rapidly stimulated ventricles have been brought into the condition above described—presenting many features of resemblance to true fibrillation but not identical in mechanism as will be explained later—diminishing rates of excitation are attended by graded changes of converse order—slower succession of contractions, less dissociation, quicker conduction, apparent coarsening of the oscillations and a gradual return, as the rate falls, to the characters of normal beats.

Pseudo-fibrillation and Fibrillation.

The above-described condition into which the ventricles may be brought by rapidity of excitation (graduated series of shocks or faradic currents of suitable strength) short of the rate necessary to induce true fibrillation, may for convenience be termed pseudo-fibrillation (figs. 5 and 6). As regards the evidence afforded by inspection, palpation, tracings of the oscillations, fall of blood-pressure, etc., the two conditions may be difficult or impossible of distinction, but they differ strikingly as regards persistence; pseudo-fibrillation ceases immediately or at varying short periods after the cessation of the stimulation, while true fibrillation in ordinary circumstances, in the absence of remedial measures, goes on as a rule to the death of the heart. (The duration of pseudo-fibrillation after cessation of the stimulation varies according to the excitability of the stimulated area, the strength and duration of the stimulating current, etc.) The difference depends on the fact that in true fibrillation a mechanism of circulating excitation has been established, whereas in pseudo-fibrillation this is not so. The latter condition depends on the emanation of an excessively rapid series of excitation waves from the area of stimulation; these short waves travelling at reduced speed over the interlaced fasciculi give rise to the condition described. But as soon as the issue of excitations from the stimulated area ceases, the disturbance ceases and the conditions revert to the normal. The pseudo-fibrillation at once ceases when the stimulated area is disconnected from the rest of the muscle, *e.g.*, by forcible clamping, etc., or when it is cut off—as may be done in the perfused heart—or when it is rapidly cooled. In pseudo-fibrillation there has not been established in the mass of the muscle outside the stimulated region a mechanism which ensures the continuance of the movement after the impulses emanating from the excited area have ceased or have been excluded—in striking contrast to what holds good in the case of true fibrillation. This method of differentiating between pseudo-fibrillation and fibrillation may be more easily applied in the case of the auricles, by isolation of the appendix after the stimulation has been applied to the tip.

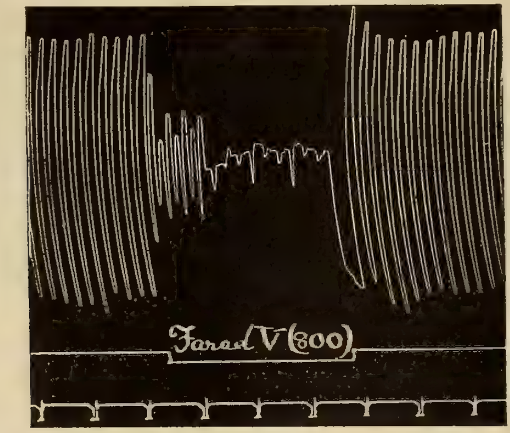


Fig. 5.—Rabbit's heart (R.V.). Faradisation with 800 units induced first a rapid tachycardia, then pseudo-fibrillation which promptly stops at the end of the faradisation. A blood-pressure record taken at the same time showed a great fall, with minute oscillations showing on the tracing.

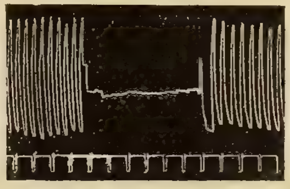
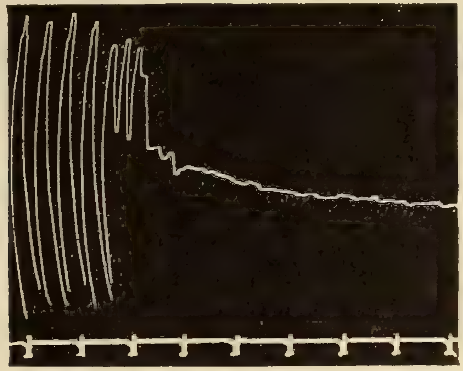


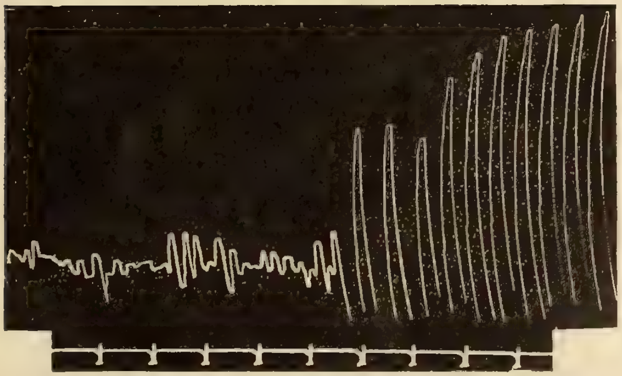
Fig. 6.—Pseudo-fibrillation induced almost immediately in fully developed form by faradisation; it ends with a larger oscillation when the stimulation ceases.

Mode of Recovery from Fibrillation.

When the ventricles are recovering from the state of typical fibrillation, with the aid of massage and of drugs, as stated later, the oscillations visible on the surface become more vigorous and clearly much coarser, the dissociation becoming much less fine and larger groups of fasciculi contracting together; there is evidently an extension of conduction through inter-fascicular junctions that were formerly blocked. On palpation the muscular substance feels of good tone, and the gradation from fineness to coarseness of fibrillation is very clearly realised—the sensation of universal turmoil due to the fine rapid dissociated twitchings throughout the ventricular walls grading into more vigorous contraction waves of coarser type, and these again into beats giving the normal feeling of uniform hardening of the muscle (figs. 7 and 8).



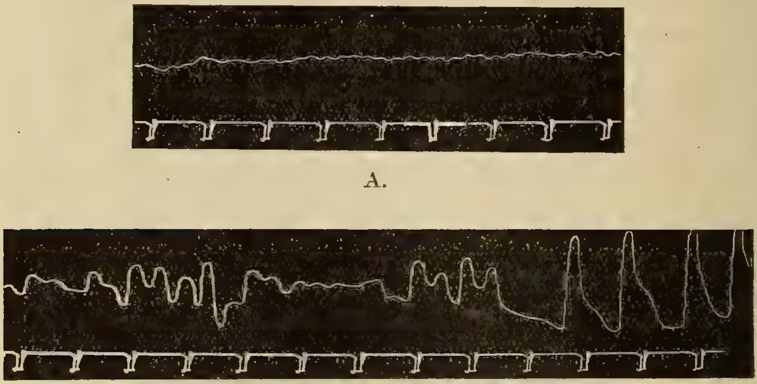
A.



B.

FIG. 7.—Spontaneous recovery from fibrillation in 30 seconds, preceded by coarsening of the fibrillar movement. Urethane, 2·5 grm., had been given hypodermically, in addition to chloroform. In A, the fibrillation was caused by shocks sent into the ventricle at the rate of 480 per minute. A brief tachycardia precedes the fibrillation. In B, recovery is seen, preceded by slower and coarser oscillations.

In the case of a heart which is showing individual fibrillar beats of the nature already described the process of recovery under the influence of massage, removal of depressing influences, etc., is usually a more elaborate one. The phase of slow coarse fibrillation has to be passed through, with a gradual increase in the rate and the grade of dissociation as excitability is restored; this leads up to the condition of rapid fine fibrillation—from which recovery occurs in the fashion stated above. But treatment with certain doses of adrenaline, etc., may sometimes change the fibrillar beats into co-ordinated ones without a transition through the various phases just enumerated (fig. 9).


A.

B.

Fig. 8.—R.V. recorded. Fibrillation from application of faradic current (200 units). A is taken 7 seconds after beginning of fibrillation. B shows recovery occurring after fibrillation had lasted 75 seconds, massage being done at intervals. Adrenaline, 0·27 mgrm., had been injected previously, and this probably favoured recovery. Marked coarsening of the movement (followed by a long pause) is seen prior to recovery.

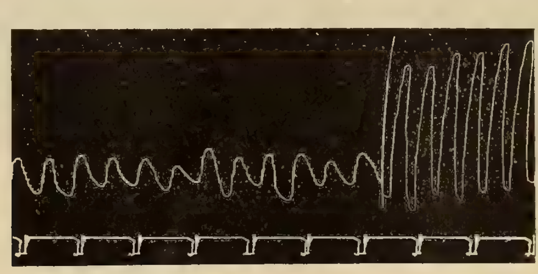

A. B.

Fig. 9.—A shows slow coarse fibrillation—a series of irregular fibrillar beats. B is taken shortly after the injection of 0·2 mgrm. adrenalin into the L.V. (1 in 5000 solution used). The fibrillar beats are changed into normal ones.

The coarsening of the rapid oscillation in the process of recovery is quite different from a coarse slow movement that is not on the way to recovery at all and where the muscle is lax and feeble. It is also different from the apparent coarsening with slowing of the oscillations in the graphic record due, as direct inspection of the heart shows, to irregular summation of fine feeble twitchings which are present with a high degree of dissociation and which may gradually become weakened to extinction. It is important to correlate the information derived from (*a*) inspection, (*b*) palpation, and (*c*) graphic records.

Rates of Stimulation Necessary to Establish the Mechanism of Circulating Excitations.

With excitable ventricles in good condition high rates of excitation by induction shocks of moderate strength are necessary to overpass the phase of pseudo-fibrillation and induce true fibrillation, *e.g.*, single induction shocks at rates of 450–500 per minute are commonly effective, but the duration of the application of the series of shocks has an influence in this respect; with longer application lower rates may suffice. When faradic currents are employed the current has to be of such a strength and duration as to raise the rate of responsive contractions to about the above rates. Beyond such rates the state of pseudo-fibrillation is not as a rule maintained, but gives place to true fibrillation as soon as the mechanism of circulating excitation has been established, this point being often recognisable on the tracing by a change from the rapid and more or less irregular curves of small excursion that are present during rapid tachycardia or pseudo-fibrillation to the much smaller and entirely irregular oscillations of true fibrillation (fig. 11).

The conductivity of the muscle plays an essential part in regard to the rate of stimulation needed to cause fibrillation; the necessary rate is not a constant or absolute one, but varies much in relation to the state of the conductivity at the time. The lower the conducting power, the lower is the rate of stimulation required to establish the circulating mechanism, since under these conditions the normal relations between conduction time and refractory period are more readily upset, a relatively low grade of acceleration sufficing to cause slowed excitation waves to reach different parts of the fascicular systems after the refractory period is over in these situations. Agents that depress conduction, *e.g.*, potassium salts, bile, cooling, etc., can be used in such a way and to such a degree as not to induce fibrillation by themselves, but to render the muscle prone to fibrillate with unusually low rates of excitation. Thus the minimal rate of stimulation which induces true fibrillation affords an indication of the state of conductivity. In conditions of greatly depressed conduction power stimuli not faster than rates commonly seen when the heart is beating in co-ordinated fashion may cause fibrillation. The rate of oscillation when fibrillation is established in such hearts is naturally a slow one, as the excitability is commonly reduced as well as the conductivity.

In such conditions of depressed ventricular conductivity, it is sometimes, though rarely, possible to excite ventricular fibrillation by faradisation of the auricles or of the sino-auricular junction in the region of the S.A. node. Such a result has been quite definitely obtained in a very few cases. The A–V. conducting mechanism was apparently able to transmit a series of impulses to the ventricles sufficient to excite in the latter the relatively low degree of acceleration necessary, in presence of their lowered conductivity, to establish the circulating mechanism.

Rates of Oscillation in Fibrillation.

As has been stated, the rates of oscillation are usually high when fibrillation is induced, and they remain high for some time; if massage is employed, quick oscillation may be maintained for an hour or more. But when, in the absence of massage, etc., the excitability of the muscle becomes lowered, as happens even with massage after a variable time under the usual experimental conditions—the rate of oscillation falls markedly, the less excitable muscle being unable to give such rapid responses to the circulating excitations. And in conditions where the excitability is depressed when fibrillation is induced, the rate of oscillation is, from the beginning, very much slower than usual; such rates as about 280, 250, 240, 140, etc., being seen, *i.e.* rates sometimes below the rhythm of a normally-beating heart when acting rapidly. It must be noted that the graphic records of the oscillations have to be interpreted with caution. For the oscillations caused by contraction waves coursing along the interlaced fasciculi are very complex and irregular and do not denote the succession of contractions in any one fasciculus. Still, the rates observed are, within certain limits, quite definite and significant, though on account of the irregularity precise figures may not be obtainable. Such records must be controlled by the methods of inspection and palpation, and, as a rule, yield results that are in accordance with the evidence afforded by the latter methods.

Influence of Duration of Stimulation.

When electrical stimulation, *e.g.*, faradisation, is used to excite fibrillation, its efficiency shows a marked relation to the duration of its application, as well as to the strength of the current; a longer application, *e.g.*, 10 seconds, may elicit persistent fibrillation when a shorter one, *e.g.*, 3 seconds, only causes a rapid tachycardia or pseudo-fibrillation. The greater effect of the more prolonged application may be ascribed to at least two factors:—

1. The time needed for the current to produce its full effect in the way of acceleration of the succession of contractions. With suitable strengths of current, the tracings clearly show an increasing acceleration for some little time after the beginning of the application, the excursions become more rapid and smaller until, when the circulating mechanism is established, fibrillation supervenes with its very irregular oscillations. With strong currents the characters of fibrillation may become manifest in the tracing immediately or almost immediately. It is evident that, with relatively weak currents, some time is needed to get up the full rate, with its influence in promoting fibrillation by shortening the refractory period and slowing and impairing the propagation of the excitations.

2. A continuance for some time of the rapid succession of contractions may be assumed to promote fatigue in the more vulnerable parts of the inter-fascicular connections (in analogy to what is known of fatigue of the A-V. conducting mechanism) by an unduly early repetition of an impulse to be conducted. Continuance of the stimulating current after the circulating mechanism has been established seems to be of no importance.

Parallelism between Auricles and Ventricles.

There are close analogies between the behaviour of the auricular and the ventricular muscular systems as regards (1) the occurrence of single contraction waves passing slowly through the muscle, constituting fibrillar beats in the ventricles, and (2) the development of (*a*) regular tachycardias, (*b*) irregular tachycardias, (*c*) pseudo-fibrillation, and (*d*) fibrillation, as results of graduated artificial stimulation.

The persistence or non-persistence of fibrillar movements is clearly explicable on the same principles in both auricles and ventricles—by the altered relation between conduction and refractory period—and the mode of conduction in fibrillation is, as in the ventricles, a fascicular one, depending on the presence of more or less extensive blocking in the inter-fascicular connections. Slow coarse fibrillation may be seen in the auricles as in the ventricles, and separate waves of contraction sweeping over the auricles in irregular fashion, more or less resembling what have been described as fibrillar beats in the ventricles, are often very striking in conditions of depressed conductivity; the progress of the greatly slowed wave can be followed by the eye with the greatest ease. And, with some increase of excitability, the wave of excitation may excite another, just as in the ventricles, and so set up a continuous slow series—slow here also because of obviously depressed excitability, as shown by diminished readiness to respond to stimuli of definite strengths.

Pseudo-Fibrillation and Fibrillation in the Auricles.

Under gradually increasing electrical stimulation, the auricles, like the ventricles, show higher and higher grades of disturbance: (1) extra-systoles, (2) regular tachycardia, (3) irregular tachycardia, (4) pseudo-fibrillation, and, at least in certain conditions of the auricular muscle, (5) fibrillation. The gradually increasing rate of auricular response rises through the grades of tachycardia or flutter, with diminishing range of lever excursions, up to a condition of rapid tremulous movement (pseudo-fibrillation), with irregular succession and range of oscillations more or less closely approximating to the characters of true fibrillation and often hard to distinguish with certainty from the latter, either by inspection of the auricles or in the tracings, though in pseudo-fibrillation the oscillations are commonly larger and of a less high grade of irregularity than in fibrillation. The movement may last for variable periods after the stimulation has been discontinued.

A ready method of discriminating between the two conditions is afforded by the experiment of isolating the stimulated area (by clamping, etc.). Tachycardia or pseudo-fibrillation is at once arrested, while true fibrillation is not affected.

In the majority of the animals examined special conditions are necessary in the auricular muscle for the production of true fibrillation with its essential mechanism by faradisation, etc., the stimulation *per se* is not, as a rule, sufficient in the easier conditions of quick conduction normally present in the auricles. Contractions in very rapid sequence, *e.g.*, 500–600 or more per minute, may be excited without establishing the mechanism of persistent fibrillation. Certain conditions involving an alteration of conductivity without a great lowering of excitability, are often effective in determining the occurrence of fibrillation, *e.g.*, vagus influence, defective blood supply, certain phases in the action of some drugs, such as chloroform, paraldehyde, pilocarpine, etc.

"Spontaneous" fibrillation, *i.e.* when the precise exciting cause cannot be defined, depends no doubt on the presence of irritation *plus* an altered state of conductivity. The latter is sometimes supplied, under experimental conditions, by the tonic influence of the vagus centre exercised through either the right or the left vagus, as can be seen when only one nerve is intact; section of the nerve in such cases is speedily followed by recovery from fibrillation which may have persisted during the whole preceding part of the experiment, or at least since the heart was exposed. Such vagus control has not appeared as a common cause of auricular fibrillation in these experiments, but in some instances its influence has been unmistakable.

The simplest and most easily available method of producing true auricular fibrillation for a time is by a combination of electrical stimulation and vagus stimulation. Rapid tachycardia set up by electrical stimulation is converted by vagus influence into true fibrillation which persists as long as the vagus influence is maintained in sufficient strength to provide the condition in the auricular musculature necessary for the keeping up of circulating excitation; the fibrillation so excited goes on under vagus influence long after the electrical stimulation has been discontinued; the latter may indeed have been applied only for a second or two. Under vagus influence the fibrillation oscillations, though very rapid, become greatly weakened, the irregular movements of the recording lever becoming minute. With pretty strong vagus control this weakening may go on to invisibility, so that the auricles look entirely quiescent, even when their surface is scrutinised with a lens. As the vagus influence wears off during prolonged stimulation of the nerve, very fine fibrillation oscillations again begin to become perceptible, and these gradually gain in vigour and range until after a variable time the normal type of beat replaces the fibrillation movement.

A similar sequence of events, more quickly passed through, is evident when vagus stimulation is diminished or discontinued instead of the influence of the nerve being allowed to wear off during continued stimulation. What evidently occurs in these cases when the auricles become motionless under vagus influence, is that the mechanism of circulating excitation goes on working in spite of the inhibitory influence which cuts down the mechanical response to invisibility; there is no true inhibition of the essential mechanism of fibrillation.

The experiment may be done in another way. Instead of first exciting the tachycardia and then stimulating the vagus, the latter may be brought into action first so as to reduce the auricles to complete quiescence; during this period an electrical current is applied briefly (*e.g.*, for one or two seconds) to the auricle; a fine tremulous (fibrillation) movement of small range may at once appear and continue until the vagus influence wanes or is discontinued.

Mechanism of Circulating Excitations without Contractions.

But if the vagus is strongly inhibiting the muscle when the electrical current is briefly applied, there may be no visible effect at all; the auricles remain perfectly motionless until the vagus control has become weakened, when the fine tremulous movement usually appears and gradually gains in vigour as in the former experiment, after a time giving place to normal action. What has happened in this case is that the electrical stimulation, falling within the period of vagus influence, is effective in setting up the mechanism of circular excitation, while the latter finds no expression in contractile movement on account of the mechanical response to excitation being kept in abeyance by the vagus inhibitory power. When the latter wanes and the mechanical response again becomes manifest, the circulating excitations are attended by the circulating contractions of visible fibrillation.

When the electrical stimulation is applied in the foregoing way without apparent effect on the inhibited auricles, the subsequent appearance and development of fibrillation as described above is not affected by the stimulated area (*e.g.*, auricular appendix) being isolated from the rest of the auricle shortly after the brief application of the stimulating current and while the auricles are still kept in complete quiescence by the vagus; the subsequent fibrillation involves the whole of the auricular muscle, apart from the isolated area. It is plain that the mechanism of excitation necessary for fibrillation has been established in the mass of the auricular muscle, and that it is independent of a continued emission of impulses from the stimulated area—now isolated. In these experiments the isolation was effected (*a*) by clamping off or (*b*) by section, after a weak clip or a ligature not too tightly drawn had been applied along the base of the appendix to prevent hæmorrhage. In some cases rapid cooling of the stimulated area was employed instead of isolation. Control experiments were made to determine that the methods used do not themselves cause fibrillation in the conditions present, under vagus influence, etc.* The vagus evidently can act more strongly on auricular contraction force, if not also on conductivity, than on excitability, for the latter property must remain functional (though depressed) in auricles that respond by subsequently manifested fibrillation movements to an electrical stimulus applied during the period of mechanical quiescence of the muscle.

As a rule, as stated above, the auricular muscle is not sufficiently depressed by vagus influence to prevent excitation occurring in response to adequate stimulation, or to stop the circulation of excitations once this mechanism has been established, though the normally-associated mechanical response may be cut down to the point of invisibility. But in some instances the vagus seems to be able to act so strongly on excitability that after electrical stimulation during the vagus period, fibrillation does not gradually appear in the usual way as the vagus control is passing off, but visible action recommences in the form of slowed auricular *beats.* This is to be ascribed to the vagus acting more strongly than usual on excitability, in addition to the usual effects on contraction force and conductivity.

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* Under certain conditions it is clear that mechanical stimulation may sometimes excite auricular fibrillation.

When the influence of the vagus in converting a rapid tachycardia or flutter into fibrillation was first studied, the question naturally arose as to whether the changes visible on inspection and in the graphic records might not be due simply to the cutting down of the force of the rapidly-recurring contractions, the mechanical limitation of the range of movement associated with distension of the auricular chambers, etc. But the clamping-off experiment brings out there is an essential difference in the mechanisms in the two cases.

The vagus alters or depresses conductivity in the auricles in such a way that the inter-fascicular connections are unable to functionate normally when the succession of excitations is much accelerated. (Distinct from this is the question of the power of the vagus to slow the conduction along the main transmitting paths in the auricles.) Certain other depressant agencies have an influence on the inter-fascicular connections in the ventricles (already described), which resembles that of the vagus in the auricles, and these agencies, when acting in great intensity, may have the further result of causing obvious and striking retardation in the passage of the contraction wave both in the ventricles and the auricles, even when the sequence is not a rapid one, but may indeed be slower than the normal.

Some Differences in the Behaviour of Auricles and Ventricles.

While the analogies between the various phenomena are very close in the auricles and ventricles, certain points of difference may be noted.

1. Electrical stimulation of strength adequate to give a sufficiently excessive rate of beat is, by itself, a ready means of exciting ventricular fibrillation, though, as has been stated, the addition of some influence depressing conductivity causes fibrillation to develop when the rate of beat is not nearly so rapid as would otherwise be required. Auricular fibrillation, on the other hand, is not, in most cases when the heart is in good condition, excited by electrical stimulation *per se,* but requires an alteration of conductivity (in the sense already defined) by some other agency, *e.g.,* vagus influence, defective nutrition, toxic substances, etc. The reason of this difference is probably to be found in conduction being less easily upset in the auricles with their simpler structure and easier conditions of rapid conduction, as compared with the highly elaborate ventricular architecture with the much slower rate of conduction in the ventricular muscle proper—apart from the Purkinje system.

2. The relation of the vagus to fibrillation is quite different in auricles and ventricles; in the auricles the vagus favours fibrillation in the presence of some irritation, *e.g.*, electrical stimulation; in the ventricles vagus influence can often be clearly shown to retard or prevent fibrillation, while not able to remove the latter once it has been established. The difference is due to the stronger action of the vagus on conductivity than on excitability, as a rule, in the auricles; this naturally promotes fibrillation. In the ventricles, on the other hand, in regard to these two properties, the main, if not the sole, incidence of the vagus influence is on excitability; this, of course, tends to repress the development of fibrillation. Pilocarpine, in suitable doses, acts similarly to the vagus, and its relation to fibrillation in auricles and ventricles is to be explained on the same lines.

3. Some drugs and toxic substances, etc., have a different incidence on the auricles and ventricles respectively both in regard to promoting and retarding fibrillation.

Confirmation of Former Views.

So long ago as 1887 the writer* put forward the view that the essential mechanism of typical fibrillation is explicable not simply as an excessive acceleration of rate *per se* or on the assumption of a mechanism of a different nature, in the sense of muscular *v.* nervous, from that concerned in the normal beat, but in a disturbance in the relation between the refractory period and the conduction time in the cardiac musculature; that when this relation is upset by shortening of the refractory period or lengthening of the conduction time or a combination of such changes, the excitation wave, in spreading over the muscular systems, reaches fibres in which the refractory period has already ended and further excitation occurs; the co-ordinated beat is thus abolished and replaced by a rapid and continued series of in-coördinated fibrillar contractions. The alteration in conduction—the passage of the slowed contraction waves in peristaltic fashion along the various complexly-arranged bundles of the ventricular wall at different points of time was described—and also the important fact that single beats may in certain circumstances be fibrillar in character.

Control of Ventricular Fibrillation.

The various actions of different agencies, in promoting or retarding the development of fibrillation and of removing it after it has been established, are to be explained by their incidence on the functions of conduction and excitability and the effects which they bring about in the relations of these functions in different conditions of the cardiac muscle (as a whole) and in the different conditions that may obtain in the auricles and ventricles respectively.

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* 'Journal of Physiology,' vol. 8, p. 296 (1887).

Any influence which depresses excitability without depressing—at least proportionately—the function of conduction naturally tends to be in some measure protective against the occurrence of fibrillation and favourable to recovery from that condition when once it has been established. A diminution of excitability opposes the attainment of acceleration sufficient to determine fibrillation; it also diminishes the responsiveness of the muscular fasciculi to circulating excitations. (The control of auricular fibrillation which differs in some respects from that of ventricular fibrillation will be dealt with elsewhere.) Similarly any agency which improves conductivity without unduly exalting excitability is inimical to the mechanism of circulating excitation. Obviously a combination of a depressing influence on excitability with the maintenance of a high level of conductivity would afford the most favourable condition for protection or recovery. Concurrent depressions or elevations of excitability and conductivity in proportionate degree naturally have no specific influence on the question of fibrillation. The agencies which operate successfully in opposing the development of fibrillation—either spontaneous (*i.e.* from unknown causes) or excited artificially by drugs, electrical stimulation, etc.—are often effective in restoring the normal action after fibrillation has been established. Remedies for fibrillation have commonly, in these experiments, been injected into the cavity of the left ventricle through the apex by means of a slender needle; sometimes intravenous injection (external jugular, etc.) was used, massage of the heart being done in both cases, while the artificial respiration is of course maintained. Smaller doses were sufficient by the intra-ventricular mode of injection. Approximately isotonic solutions were used, warmed to body temperature. The doses stated are for cats, usually weighing 2–3 kilos. but sometimes more.

Urethane.—Doses varying between 0·025 and 0·25 grm. injected into the left ventricle were found effective in removing fibrillation in very numerous experiments (fig. 10); 3 per cent. solutions were commonly used for

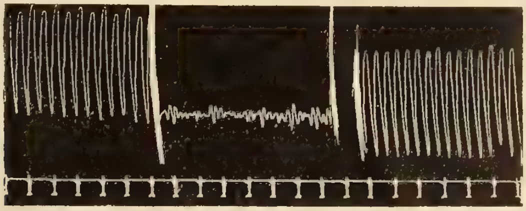

Fig. 10.—The middle portion of the tracing shows fibrillation caused by strong faradisation (5000 units). After it had lasted for 2 minutes (with occasional massage) 0·05 grm. urethane was injected into the L.V. The restored action is seen in the right-hand portion.

intra-cardiac or intra-vascular injections. Hypodermic doses of 0·5 grm. per kilogramme and upwards (given in 25-per-cent. solution, etc.) have a pronounced influence in protecting against fibrillation in light chloroform anæsthesia and in diminishing, though not always obviating, the danger of adrenaline fibrillation in the same grade of anæsthesia. Sufficient time has to be allowed for absorption before the effects are tested. Smaller doses suffice for this purpose when given by intra-vascular (*e.g.*, saphenous vein) injection.

Strontium Chloride was given in doses of 0·01—0·06 grm., a 1-per-cent. solution in dilute Ringer's fluid being usually employed.* Especially when applied at an early phase of the fibrillation this remedy often succeeded very well, and the condition of the heart and circulation were excellent afterwards (fig. 11). In other cases after fibrillation had lasted for a long time and other measures had been unsuccessful, this salt sometimes speedily induced recovery. Fibrillation, in its various phases, caused by potassium salts is, as might be expected, specially amenable to treatment with strontium in doses varying according to the toxic dose of potassium.

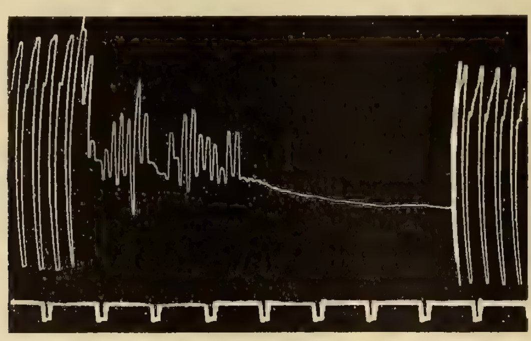
A. B.

Fig. 11.—A, fibrillation, preceded by period of tachycardia and pseudo-fibrillation, from faradising with 500 units. Injection of 0·06 grm. strontium chloride was followed in 30 seconds by restoration of the normal action, shown in B, taken shortly after recovery. Soon afterwards faradisation with 1000 units again caused fibrillation; recovery followed injection of 0·03 grm., with the usual massage.

Adrenalin.—Solutions of 1 in 10,000 or 1 in 5,000 were commonly used; sometimes as strong as 1 in 1,000; in Ringer's fluid in each case. The dose varied from 0·1 to 1 mgrm. Successful results were very frequent in fibrillation which had been induced in various way—by electrical stimulation, chloroform, adrenalin injection during light chloroform anæsthesia (the chloroform-adrenalin reaction described by Levy and abundantly illustrated in this investigation), intravenous injection of potassium salts, etc. In many instances fibrillation has been induced by a small dose (*e.g.*, 0·1 mgrm.) of adrenalin and remedied by the intraventricular injection of a very large dose (up to 1 mgrm.), the state of the heart and circulation remaining good afterwards (fig. 12). The excitability and conductivity of the muscle are enhanced by a small injection and as early effects of a large injection; subsequently a pronounced depression of excitability occurs—shown in many cases by a great diminution in responsiveness when tested by graduated faradic currents; stimulation, that formerly induced fibrillation readily, now fails to do so even when strengthened to many times its former intensity. Diminished sensitiveness to faradic currents is often pronounced, while the blood-pressure is still elevated and the heart is beating very strongly. Adrenalin can thus act in two ways: (*a*) by reducing excitability, and (*b*) by improving conduction.

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* The amounts here stated are of strontium chloride crystals ($SrCl_2 + 6H_2O$). The doses of the anhydrous salt would be represented by about 60 per cent. of the above amounts.

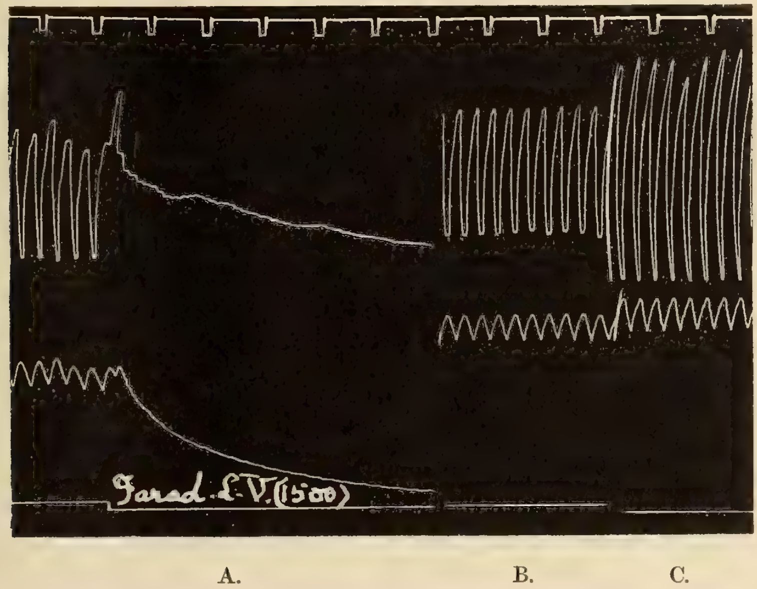

Fig. 12.—The upper tracing is from the left ventricle, the lower indicates the blood-pressure. In A, fibrillation caused by faradisation with 1500 units lasted 6 minutes, recovery following injection of 0·5 mgrm. adrenalin in three doses. B is shortly after recovery. C, taken 1 minute later, shows much increase in the range of the lever excursions. Note that the blood-pressure is still elevated.

Hirudin.—Injections* (into the saphenous vein) of about 8–10 mgrm. per kilogramme of body-weight showed striking effects in opposing the development of fibrillation, either "spontaneously" or in response to electrical stimulation, etc. Even powerful faradisation (often several thousand units) caused only a pseudo-fibrillation, ceasing almost immediately or lasting only a short time (seconds) after the stoppage of the current, or a true fibrillation, which is spontaneously recovered from—on account of the diminished responsiveness of the muscle to the circulating excitations.

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* Doses of 0·3–0·5 mgrm. were often effective in removing fibrillation injected into the L.V. The solution of hirudin used generally contained 1 mgrm. in each cubic centimetre of Ringer's fluid.

Pilocarpine.—Intravenous injection (into jugular, etc.) of 0·0025 grm. (with massage of the ventricles) was often effective in arresting ventricular fibrillation. There was a good deal of variation in regard to this result; there seemed to be a parallelism between the efficiency of pilocarpine in this respect and the activity of vagus inhibition in the particular heart in question—as tested by stimulation of the vagus in the neck or, preferably, the inhibitory area on the dorsal aspect of the auricles. Though vagus stimulation has not been found to arrest fibrillation once it has been established, it has shown notable effects in opposing the development of fibrillation in certain circumstances. And pilocarpine is much more potent than the vagus, though its influence is in the same direction and of the same nature in many respects at least.

Similar remedies were found applicable to the perfused heart, also, a little of the solution of urethane, adrenalin, etc., being injected into the tube leading to the aorta; very small doses usually sufficed.

In some instances, where ventricular fibrillation does not yield so readily as usual to a single remedy, combinations such as urethane and adrenalin, or these followed by strontium chloride, prove very effective. After such treatment the ventricles commonly show a remarkably great resistance to electrical stimulation as far as the induction of fibrillation is concerned, very powerful currents up to 7,000–10,000 units, etc., often causing only pseudo-fibrillation, and, if true fibrillation, with its special mechanism, is induced, it very frequently shows spontaneous recovery after variable periods, frequently without any massage or with massage for some seconds. The difficulty in exciting fibrillation, and its notable tendency to recover, are often very striking, and are to be accounted for, in the main at least, by the diminished responsiveness of the muscle induced by the drugs.

Some relations of different remedial agents to special conditions of the heart may be noted. In very excitable hearts that have fibrillated, depression of excitability is the primary requirement. On the other hand, when direct depression of conductivity (*e.g.*, by potassium salts, bile, cooling, etc.) is the predominant factor in any particular heart, remedies calculated to enhance this function are obviously indicated, whether they act (*a*) by

PROCEEDINGS
OF
THE ROYAL SOCIETY.

Series B. Vol. 90. No. **B 630.**

BIOLOGICAL SCIENCES.

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CONTENTS.

Page

The Artificial Production of Echinoderm Larvæ with Two Water-Vascular Systems, and also of Larvæ Devoid of a Water-Vascular System. By E. W. MACBRIDE, F.R.S. (Plates 4–10) 323

Changes of Electrical Conductivity under Geotropic Stimulation. By JAMES SMALL, M.Sc. (Lond.), F.L.S. 349

PRINTED AND PUBLISHED FOR THE ROYAL SOCIETY BY
HARRISON & SONS, 44–47, ST. MARTIN'S LANE, LONDON, W.C.2.
Price Four Shillings and Sixpence.

No. B 630.

December 20, 1918.

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direct improvement of conductivity or (*b*) secondarily through the slowing of the rate of succession which they may induce, *i.e.* by lowering excitability, provided that this effect is not attended by a proportionate lowering of conductivity. Adrenalin is notably useful in this respect, as indicated by the remarkable improvement in conduction often seen under its influence, especially evident in the auricles, where a strikingly slow contraction wave, present during gravely depressed conduction, may be replaced by an approximation or a return to the normal type. Hence the special utility of adrenalin in dealing with forms of slow coarse fibrillation, already described, and also with fibrillar beats—unless the damage in the latter case has been carried to an irreparable stage (fig. 9).

The success of the above-mentioned methods of obtaining recovery from typical fibrillation, induced by means that did not permanently damage the heart, has been such that in recent years of experimentation there has not been failure in any instance.

For valued assistance in some of the experiments of this investigation, I have to record my thanks to Drs. G. Spencer Melvin and J. R. Murray.

A portion of the costs was defrayed by a grant from the Carnegie Trust.

The Artificial Production of Echinoderm Larvæ with Two Water-Vascular Systems, and also of Larvæ Devoid of a Water-Vascular System.

By E. W. MacBride, F.R.S.

(Received January 18, 1918.)

[Plates 4–10.]

The development of Echinoderms has been characterised, and with justice, as the most remarkable ontogenetic change in the animal kingdom. For the larva is an almost perfect example of a simple, bilaterally symmetrical Metazoon, and the amazing thing is, not that the radially symmetrical adult should develop out of a bilaterally symmetrical larva, but that the axis of symmetry of the radial adult should cut the principal axis of the bilateral larva at an angle which approaches 90°.

In the three orders Asteroidea, Ophiuroidea, and Echinoidea, the general anatomy of the early larva is of the same type. In all three groups the larva possesses a simple alimentary canal, consisting of a conical œsophagus opening by a wide mouth, a globular stomach, and a sac-like intestine opening by a narrow anus and directed forwards, so that the whole alimentary canal has the form of a **U**. On each side of the œsophagus a flattened cœlomic sac is situated; of these, the left sends up a vertical outgrowth termed the **pore-canal,** which fuses with the dorsal ectoderm, and opens to the exterior by a pore called the **madreporic pore.** Each cœlomic sac subsequently grows backwards, so that its posterior portion lies beside the stomach, and this portion later becomes separated by a constriction from the rest. Consequently, each sac becomes divided into an **anterior** and a **posterior cœlom.**

In all three orders, when this stage has been reached, a vesicle is budded off from the posterior end of the anterior cœlom on the left side, which is termed the **hydrocœle.** This vesicle dominates the whole later development of the larva, and eventually becomes transformed into the water-vascular system of the adult. It gives off lobes, or **primary tentacles,** which become converted into the radial vessels of that system, and it becomes bent into the form of a hoop, which is then converted into a ring by the meeting of the two ends, and this ring is the water-vascular ring-canal. The axis of adult symmetry, as all know, passes through the centre of this ring. At metamorphosis, portions of the larval body are cast off, consisting mainly of the ciliated band, with its various outgrowths or "arms," which constitute the organs of locomotion of the larva. The left side of the larva becomes converted into the ventral or actinal side of the adult which bears the tube-feet, whereas the right side of the larva becomes changed into the dorsal or abactinal side of the adult, developing spines and pedicellariæ as the case may be.

It is obvious that, if not only the *left*, but also the *right*, anterior cœlom, were to bud off a hydrocœle, the bilateral symmetry of the larva would be retained, and the whole subsequent course of the development would be profoundly modified.

Johannes Müller figured, without describing it, an Ophiurid larva, with a five-lobed hydrocœle on both sides of the œsophagus (16). Later, Metschnikoff (15), who investigated the development of the viviparous Ophiurid *Amphiura squamata*, asserted that the rudiment of a hydrocœle was normally formed on each side of the embryo, but that only the left rudiment developed.

In 1895, when engaged in the investigation of the development of the Asterid *Asterina gibbosa*, I encountered several specimens with two hydrocœles, and these were described in my paper on the development of that species (11).

In 1910 my friend and colleague, Mr. Fuchs, whilst making cultures of the larvæ of *Echinus esculentus*, discovered one with two hydrocœles. This abnormal specimen he was good enough to hand over to me for examination, and in 1911 I published an account of it (14), describing at the same time a similar specimen of the larva of *Echinus miliaris*, which I had found in my own cultures. In 1911 a larva with two hydrocœles was observed by Caswell Grave in cultures of the larvæ of the Clypeastrid *Mellita testudinata*, and a similar larva was found by von Ubisch in 1913 in cultures of the larvæ of *Strongylocentrotus lividus* (18). Finally, in 1914, Gemmill published a description of a number of larvæ with two hydrocœles, which he discovered in his cultures of the larvæ of the Asterid *Asterias rubens* (6). In this paper Gemmill suggests that the doubling of the hydrocœle was due to some factor in the environment, because practically all the larvæ in one jar showed signs of two hydrocœles. In 1915 he also recorded the occurrence of larvæ with two hydrocœles in cultures of the larvæ of *Porania pulvillus* (7).

In this list of recorded instances of the occurrence of two hydrocœles amongst Echinoderm larvæ, it will be noticed that only one such specimen was found amongst larvæ fished from the open sea, although hundreds of such larvæ have been examined. This unique larva was that figured by Johannes Müller. All the others were found amongst larvæ reared in confinement, and this circumstance led me to agree with Gemmill in ascribing the formation of the double hydrocœle to some factor in the environment. Gemmill put forward the tentative suggestion that over-feeding caused the formation of two hydrocœles amongst Asterid larvæ, for he noticed that, in the jar in which the largest number of larvæ with two hydrocœles was found, the diatom (*Nitzschia*) which he used as food for them had undergone great multiplication. In the process of metamorphosis amongst Asterid larvæ, the partitions between the various cœlomic cavities become to a large extent absorbed, and, in particular, the left posterior cœlom sweeps to the right under the stomach, and opens into the right anterior cœlom. In overfed larvæ, according to Gemmill, the swollen stomach prevents this process from taking place, and the right anterior cœlom, freed from the disturbing contact with the left posterior cœlom, develops in the same way as its left antimere, *i.e.* it buds off a hydrocœle from its hinder end.

It is worthy of note that Gemmill had previously observed (5) that in about 10 per cent. of the young larvæ contained in a normal culture of the larvæ of *Asterias rubens* the right anterior cœlom formed a pore-canal, so that there were two pore-canals and two madreporic pores, a right and a left, but there was no relation to be found between the occurrence of two pore-canals and two hydrocœles. Most of the larvæ with two pore-canals possessed only one hydrocœle, and some of the larvæ with two hydrocœles had only one pore-canal.

The cause assigned by Gemmill for the production of two hydrocœles, even if correct, could only be operative in the case of the larvæ of Asteroidea, for at the stage of development at which two hydrocœles have been observed in the larvæ, both of Ophiuroidea and of Echinoidea, no such extension of the left posterior cœlom normally takes place; indeed, at no time in the normal development of the larvæ belonging to these two orders does this cavity come into relation with, much less open into, the right anterior cœlom.

As the larvæ of the common green shore urchin *Echinus miliaris* had for several years been successfully reared through their entire development in the tank-room of the Zoological Department of the Imperial College of Science, it seemed to me that this species would form a very suitable subject on which to investigate the causes of the production of a double hydrocœle. These investigations were begun in the summer of 1914, and carried to a successful conclusion in the summer of 1917.

MATERIAL AND METHODS.

The specimens of *Echinus miliaris* used in these experiments were procured from Plymouth in 1914, from the Essex coast in 1915 and 1916, and in 1917 from Looe, in Cornwall. All four lots gave concordant results, and so the conclusions at which I finally arrived have a considerable body of evidence behind them. The best results were obtained from the Looe sea-urchins, and I have to thank Dr. Allen, the Director of the Plymouth Marine Biological Station, for his kindness in sending a special expedition to Looe Island in order to secure these specimens.

The urchins were sent to London by the swiftest route available, packed in damp seaweed. A laboratory assistant awaited their arrival at the railway terminus, so that the delay which would have been occasioned by the slowness of delivery in London was entirely obviated. They were opened and the ova and sperm were extracted from them, within an hour of their arrival at the laboratory. The sea-water used in order to carry out the fertilisation of the eggs and the rearing of the larvæ was sterilised by the method introduced by Dr. Allen in Plymouth. This method is as follows:—The sea-water is first shaken up with animal charcoal and then allowed to settle and the clear fluid is siphoned off. This fluid is in this way freed from soluble toxins which might interfere with the growth of the larvæ. It is then passed through a Berkfeld filter in order to remove from it all bacteria.

The ripe eggs obtained from the females were fertilised in finger bowls, and the next morning, when the free-swimming blastulæ had risen to the top these were decanted off into "Breffit jars" in which a considerable portion of, and in some cases all, their further development took place.

These "Breffit jars"—the use of which was introduced by Dr. Allen and the workers in the Plymouth Marine Biological Laboratory—deserve some words of comment. They are made of thick green glass, and each contains, when full, about 2½ litres of sea-water. The colour of the glass, which admits approximately only the same rays of light as can penetrate the surface-layers of the sea, seems to be of some importance in keeping the larvæ healthy. The jars were placed in a sink which was kept moist by a trickle of water from the tap.

The larva is capable of developing for four or five days without food, indeed the ectodermal œsophagus only joins the endodermal stomach on the fourth day (fig. 1). By the fifth or sixth day the initial impetus of development has been exhausted, and although the larva can live for several days longer, degenerative processes set in, and sooner or later it falls to the bottom and dies. The degeneration shows itself especially in the absorption of the ciliated arms.

It is therefore necessary to supply the larva with food, and the food chosen was a pure culture of the diatom *Nitzschia*. These pure cultures are prepared in the Plymouth Biological Station, and directions for sub-culturing are given in Dr. Allen's paper on the subject (1).

The successful rearing of the larvæ depends principally on the establishment of a healthy growth of *Nitzschia* in the culture jar; but according to Dr. Allen's experience, not more than about thirty larvæ will grow to an advanced stage of development in a Breffit jar, and of these ordinarily only a few will complete their metamorphosis. It is, however, possible to rear hundreds of larvæ in such a jar until they are about a fortnight old. But for a successful issue of a rearing experiment, it is necessary as the larvæ grow older to thin out the culture at frequent intervals by transferring portions to fresh Breffit jars, and my experience coincides with that of Dr. Allen that one is lucky if one succeeds in rearing six or seven larvæ in each jar through the metamorphosis.

In 1914, 1915, and 1916 I reared the larvæ in Breffit jars, but I was dissatisfied with the numerical results obtained, and in 1917 I adopted the plan of transferring the larvæ when they were about ten days old, into much larger receptacles, viz., the plunger jars.

A plunger jar, such as is installed in the tank-room in the Zoological Department of the Imperial College of Science, is a large glass bell-jar which holds when inverted about 30 litres of water. Each jar is provided with a glass cover, the upper side of which is covered by black paper, and in this way the water contained in the jar is shielded from the direct action of sunlight, as the tank-room is lit from above. The plunger was invented by Mr. E. T. Browne, and is described by him in a paper on the rearing of Medusæ (2). It keeps the water in constant slow movement.

If one compares advanced larvæ reared in plunger jars with those reared in Breffit jars, one is struck with the fact that the former have far longer arms than the latter ,and in this respect are certainly more normal, for they resemble the larvæ that are found in the open sea. But the production of more normal larvæ is by no means the only advantage which accrues from the use of plunger jars. A much more important one is the far greater number of larvæ which can be successfully reared to an advanced stage of development. In one of these jars at the Imperial College, on a previous occasion, no less than 200 larvæ completed their metamorphosis, and in 1917, when the experiment was only carried on until the larvæ were six weeks old, one plunger jar alone must have contained at least 1000 larvæ. If one contrasts this result with that which is obtained by rearing the larvæ in Breffit jars, the advantage of the plunger jar becomes obvious. Even if it were possible—which it is not—to rear to an advanced stage 50 larvæ in each Breffit jar, one would require 20 such jars containing in all 50 litres of water in order to accommodate 1000 larvæ; but, as every experimenter knows, it is practically impossible to secure exactly the same conditions in any two Breffit jars; in some the *Nitzschia*, for some unexplained reason, fails to flourish, and as a consequence few larvæ survive. For this cause, whilst I was able to obtain qualitative results in 1914, 1915, and 1916, quantitative results were only obtained in 1917.

The larvæ of *Echinus miliaris* are beautifully transparent, and were in many cases drawn living. Very serviceable whole mounts were obtained by preservation in carefully neutralised 40-per-cent. formalin, followed by absolute alcohol. Alcoholic eosin or light green was used for staining.

In order to elucidate the relationship between the right hydrocœle and the pore-canal it was necessary to cut sections. These were taken parallel to a plane cutting the larval arms at right angles. Larvæ of which it was desired to cut sections were not preserved in formalin, as this fluid does not give sufficiently good fixation of histological details. They were preserved in Bouin's fluid, which gives a very good preservation, and which makes the larvæ far less brittle than osmic acid followed by Müller's fluid, which I employed in previous work on Echinoderm larvæ. They were embedded in celloidin, and subsequently in paraffin, by the method which I described in detail when giving an account of the development of *Echinus esculentus* (12).

THE NORMAL DEVELOPMEMT OF ECHINUS MILIARIS.

In a former communication to this Society (12), I have described in detail the development of *Echinus esculentus*, and *Echinus miliaris*, the larva of which was first described by me in 1898 (11), develops in essentially the same way. Nevertheless, it seems desirable to recapitulate briefly the principal points in my paper on the development of *Echinus esculentus*, in order that the reader may more easily grasp the results of the experiments to be described in this paper.

The result of the segmentation of the egg is to form a spherical **blastula,** one side of which then becomes flattened, and an invagination of the centre of this flattened surface forms the **primordial gut** or **archenteron,** and so the blastula is changed into **a gastrula.** From the apex or blind end of the archenteron a bilobed vesicle is cut off; this is the rudiment of the **secondary body-cavity** or **cœlom;** the wide space which intervenes between the archenteron and the ectoderm, and which contains a few connective tissue cells, is the **blastocœle** or **primary body-cavity** (fig. 1).

The larva now grows in length, and becomes obliquely flattened on its anterior surface; it is as if it were bevelled. This new flattened surface is the **oral surface,** and in the midst of it the mouth is formed as the opening of a wide shallow invagination, which is termed the **stomodæum.** The archenteron having given off the cœlomic rudiment now becomes converted into the **larval gut.** It was originally straight, but it now becomes arched, so that its apex is bent towards the bottom of the stomodæum, and it becomes divided by constrictions into three portions, viz., **œsophagus, stomach,** and **intestine.** The oral surface of the larva becomes surrounded by a thickened ridge of ectoderm, which carries very long and powerful cilia, and so the **ciliated band,** which is the organ of locomotion of the young larva, is formed.

Soon after, the bottom of the stomodæum breaks through into the œsophagus, and so the formation of the larval gut is completed. The **anus** is, of course, the persistent **blastopore** or opening of the invagination which made the archenteron. The cœlomic rudiment becomes divided into **right** and **left cœlomic sacs** at the same time, and the ciliated band grows out into four lobes. These lobes are the first of the **larval ciliated arms** to be formed; two are situated in front at the sides of the mouth and are termed **antero-lateral,** and two are placed behind the mouth in front of the anus. These latter are termed **post-oral arms.** The two arms on each side of the larva are supported by delicate calcareous rods which are outgrowths of a calcareous **star,** the beginnings of which can be traced back to the blastula stage. On the 5th day the left cœlomic sac sends out a tubular protrusion, which ascends to the dorsal surface, fuses with the ectoderm, and opens to the outside. This outgrowth is the **pore-canal,** and its opening to the exterior is the **primary madreporic pore.**

The development up to the point which I have just described is exceedingly familar to all workers in experimental embryology, for *Echinus miliaris* is closely allied to the famous *Echinus microtuberculatus* of the Bay of Naples, if indeed it be not merely a northern race of that species. As all know, countless experiments have been made on the larva of *Echinus microtuberculatus,* but few experimenters have carried their cultures of the larvæ beyond the point of development which has just been described, which is just the point at which the larvæ begin to require food.

As mentioned above, the appearance of the older larva was first described by me (12) and the only series of experiments in which the older larva has been used are those carried out by Debaisieux (3) in the laboratory of the Imperial College under my supervision and those made in the marine biological laboratory at Plymouth by Shearer, De Morgan and Fuchs (16).

When the larva has attained the age of about eight days the left cœlomic sac becomes divided into anterior and posterior portions, and a little later the right cœlomic sac begins to be constricted into anterior and posterior portions (fig. 2), but these parts are not finally divided from one another until the 12th day. On the 10th day the **hydrocœle** begins to be formed as a swelling of the hinder end of the left anterior cœlemic sac. This bud-like swelling is never completely separated from the rest of the sac; the neck of union between the two portions becomes drawn out so as to form a narrow tube, which is the **stone canal** (*st.c.*, fig. 4). *It follows that the connexion of the hydrocœle with the exterior is an indirect one, for the left anterior cœlom persists into the adult stage as a sac-like space termed the* **axial sinus,** *and the stone canal leads from the hydrocœle or water vascular ring into this sinus, and from this sinus the pore-canal (which in the adult becomes multiplied into numerous pore-canals) leads to the exterior.*

The ciliated band grows out on each side into an extra arm termed the **postero-dorsal arm**, which is supported by a calcareous rod growing out from a new calcareous star on each side. In the bay surrounded by the loop of the ciliated band leading from the base of this arm to the base of the post-oral arm, an invagination of the ectoderm takes place which is termed the **amniotic invagination** (*am.*, fig. 3). The ectoderm at the bottom of this pit becomes thickened and pressed against the hydrocœle bud, whilst the opening of the pit becomes narrowed and eventually closed: the pit is then termed the **amniotic sac** and its roof the **amnion.**

The complex structure consisting of the adpressed hydrocœle and floor of the amniotic sac is known as the **Echinus-rudiment,** and the subsequent development of the larva consists mainly in the growth and elaboration of the Echinus-rudiment (*Ech.*, fig. 5).

Before, however, we deal with the further development of this structure, we may glance at the changes which take place in other parts of the larva. A fourth pair of arms which are termed the **præ-oral arms** grow out from that portion of the ciliated band which extends between the bases of the two antero-lateral arms. This pair of arms is extremely apt to be imperfectly developed in larvæ which have been artificially reared; most frequently one of the pair is longer than the other, but sometimes one and sometimes even both remain entirely undeveloped. They are supported by calcareous rods which are outgrowths of a median Y-shaped ossicle the **dorsal arch** (*d.a.*, fig. 3), which lies above the œsophagus and which makes its appearance about the 18th day, before indeed any trace of the arms themselves has appeared. The stem of the Y extends backwards and develops into a calcareous network which surrounds the madreporic pore and is the rudiment of the **madreporic plate** or **madreporite** of the adult. From this plate about the 30th day a knob sprouts out which becomes one of the adult spines and is termed by me the **madreporic spine** (*m. sp.*, fig. 22).

About the 15th day the **ciliated epaulettes** make their appearance. These rapidly develop into great crescentic lobes, horizontally placed, which together almost encircle the larva. They bear numerous long cilia and in later larval life constitute the most important locomotor organ of the larva. They were originally loops of the ciliated band, the dorsal ciliated epaulettes being formed from the loops between the antero-lateral and the postero-dorsal arms, whilst the ventral epaulettes are formed from that part of the ciliated band which stretches between the bases of the post-oral arms. These loops become entirely separated from the ciliated band, but the breaches of continuity in the band so caused quickly heal up (*d.c.ep.*, *v.c.ep.*, fig. 5). So far as is yet known, ciliated epaulettes are confined to comparatively few genera and are not by any means characteristic of all Echinoid larvæ.

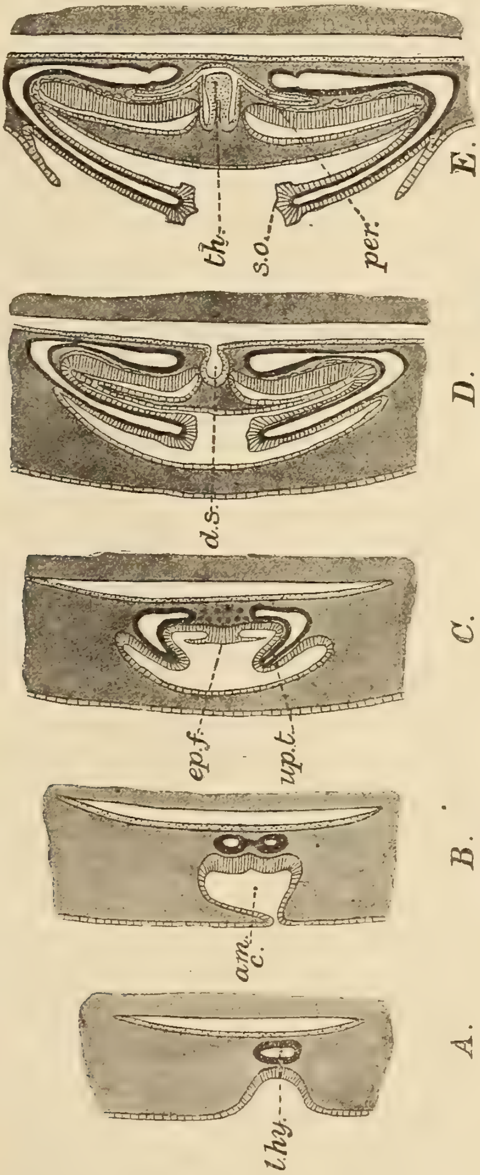
We have mentioned above that the separation of the right anterior cœlom from the right posterior cœlom is not completed before the 12th day. After it is complete the right anterior cœlom sends out from its posterior end a tongue of cells which arches over the œsophagus towards the mid-dorsal line. From the apex of this tongue a cellular bud is detached, which becomes hollow and constitutes the **madreporic vesicle,** so termed because it lies just under the madreporite close to the madreporic pore. In my paper on the development of *Echinus esculentus* (12) I put forward the view that the vesicle represented a vestigial right hydrocœle. At that time I had never seen an *Echinus* larva showing a genuine right hydrocœle. It will be made plain in this paper that that interpretation was incorrect, for the madreporic vesicle may be well developed in larvæ which show an unmistakeable right hydrocœle (figs. 18, 21).

On the right side of the larva, about opposite the spot where the amniotic invagination is formed on the left side, just above and below the loop of the ciliated band which extends between postero-dorsal and post-oral arms, two knobs begin to appear about the 20th day. About the 25th day it becomes obvious that each is developing into a **pedicellaria.** Each is based on a calcareous plate which becomes one of the five genital plates of the young sea-urchin.

As the larva grows older an additional pedicellaria and several square-topped spines appear on each plate. These plates are just as characteristic of the right side of the larva as the Echinus-rudiment is of the left side (fig. 6); midway between them another calcareous plate is developed (*calc.*, fig. 6).

We may now consider the development of the Echinus-rudiment. This structure, originally small, grows until it occupies the whole side of the larva. The hydrocœle gives rise to five lobes which protrude into the amniotic space, each covered, of course, with a layer of ectoderm derived from the floor of the amniotic sac. These lobes are the **five primary tentacles** which constitute the radial water-vascular canals of the adult. It is characteristic of *Echinus miliaris* that each primary tentacle soon gives rise to two lateral buds which constitute the first paired tentacles, whereas in *Echinus esculentus* this event does not take place until after metamorphosis. Between the bases of each two adjacent primary tentacles there are developed as outgrowths of the floor of the amniotic sac four pointed spines, each of which soon develops at its base the neuro-muscular collar characteristic of the adult spine. This area of the amniotic floor, between two tentacles, which gives rise to these spines may be termed the **interradius.** From the outer wall of the left posterior cœlom lying beneath each interradius, a pocket-like evagination is formed. Each pocket becomes one of the **dental sacs** (*d.s.*, fig. 19) of the adult and in its walls calcareous ossicles are formed which give rise to a tooth and the pair of jaws embracing it. The five dental sacs and the ossicles to which they give rise constitute the complex structure known as Aristotle's lantern in the adult. After metamorphosis an outgrowth from the larval stomach meets a central depression of the amniotic floor and in this way the adult mouth and œsophagus are formed.

It will be obvious, therefore, that in the formation of the Echinus-rudiment no less than three distinct tissues are involved. These are (1) the hydrocœle, (2) the amniotic floor, and (3) the wall of the left posterior cœlom. One of the main objects of the experiments described in this paper was to determine,



Text-figure.—Illustrating the Growth of the Echinus-rudiment.

am.c., amniotic cavity; *d.s.*, incipient dental sac; *ep.f.*, epineural fold; *l.hy.*, left hydrocœle; *per.*, perihæmal canal growing out from the dental sac; *s.o.*, sense organ in apex of primary tentacle; *th.*, tooth rudiment in dental sac.

if possible, how far these tissues were independent of one another in their modifications, or whether the modification of one caused the modification of the rest.

DESCRIPTION OF THE EXPERIMENTS.

A. *The Production of Larvæ with Two Hydrocœles.*

On thinking over the possible changes in the environment which might be likely to be encountered by larvæ reared in artificial cultures it occurred to me that the salinity of the water in the culture-jar might be raised by accidental evaporation and that this stimulus might be the factor which caused the formation of a second hydrocœle. I may say in anticipation that this surmise proved to be partly right and partly wrong. It is true that increased salinity, acting at a certain critical period of development, will lead to the production of a right hydrocœle, but other causes must also be competent to produce the same result, for increased salinity will not account for every instance of this phenomenon.

It transpired, from preliminary experiments in 1914, that larvæ would grow fairly well in water of which the salinity had been raised by evaporation to 37 parts per thousand, and with difficulty in water of which the salinity was 39 parts per thousand. Accordingly, for all further experiments, water of a salinity of 37 parts per thousand was employed, although in 1914 some trials were made of transferring larvæ which had been reared up to a certain stage of development in water of a salinity of 37 parts per thousand to water of a salinity of 39 parts per thousand. Water of enhanced salinity is usually denoted as **"hypertonic,"** and, for the sake of brevity, I shall adopt this term in further discussion of the subject.

I was unsuccessful in fertilising the eggs in hypertonic water, although I do not assert dogmatically that this difficulty could not be overcome. However, I abandoned further attempts to do this, and all the eggs used in these experiments were fertilised in normal sea-water, and the larvæ were transferred to hypertonic water when they had reached an age of three days (1915, 1916) or four days (1917). The exact stage of development attained at these ages varies with the time of year and with the vigour of the culture, but, broadly speaking, the stage reached at four days may be taken to be characterised by the completion of the alimentary canal, by the division of the cœlomic rudiment into two equal cœlomic sacs, and by the appearance of the antero-lateral and post-oral ciliated arms, but by the absence of a madreporic pore. In a word, the stage may be described as one in which *no departure from strict bilateral symmetry has as yet occurred* (fig. 1).

In 1914, when the larvæ were about a fortnight old, and had lived for

10 or 11 days in the hypertonic water, the hydrocœle was formed on the left side as in normal cultures, *but in some larvæ there appeared on the right side an outgrowth from the right anterior cœlom in the form of a little vesicle, which was not present in normal larvæ* (*r.hy.*, fig. 3). I hailed this structure as a rudiment of a right hydrocœle, but no amniotic invagination was formed over it, and, in spite of all my endeavours, the larvæ refused to develop further, and so this experiment led to an indecisive result.

In 1915 the experiment was repeated, and the same result was obtained. Some of the most promising larvæ were picked out and placed each in normal sea-water in a separate finger-bowl, with a rich supply of *Nitzschia*. In one of these larvæ the bud on the right side developed five lobes, showing that it really was a right hydrocœle; the other specimens died.

In 1916 the experiments were again repeated, and, during that year, some larvæ were kept living in Breffit jars filled with the hypertonic water, whilst others, after having been for periods varying from a week to three weeks in this water, were transferred to Breffit jars filled with normal sea-water. At the end of six weeks I had the immeasurable satisfaction of seeing some larvæ belonging to both categories develop an unmistakable right hydrocœle, provided with five tentacles, which projected into a well-formed amniotic cavity (fig. 7). In these experiments, the tendency of the hypertonic water to inhibit the growth of the *Nitzschia* was overcome by the constant addition of fresh doses of *Nitzchia*.

I was still, however, far from satisfied with the results which I had obtained. The larvæ grown in the Breffit jars were in some respects unhealthy, as fig. 7 shows, and the number which survived until reaching the stage when the hydrocœle develops lobes was small, and so I made arrangements to repeat the experiment on a large scale during the summer of 1917.

Owing to difficulties connected with the war, I was unable to secure a supply of sea-urchins until July 7. The experiment was closed on August 14, and so it lasted only between five and six weeks—an insufficient amount of time to allow of the larvæ completing their metamorphoses, but long enough to allow of them producing large Echinus-rudiments with all their characteristic features. As I have already mentioned, the urchins sent me in this year (1917) were collected at Looe Island, and I have reason to believe that, owing to the experience of the assistants of the Plymouth Laboratory, who packed and forwarded them, they reached me in better condition than the urchins which I received in 1915 and 1916.

I decided to increase the salinity of the water, not by evaporation, but by the addition of 5 grm. of common salt to each Breffit jar, which raised the amount of all the salts present in the water to 37 parts per thousand. When the method of evaporation is used, the concentration of all the salts present in sea-water is raised in equal proportions, and, in addition, the acidity of water is reduced by the expulsion of CO_2. In the method employed in 1917, the acidity of the water was undisturbed, and the concentration of NaCl alone was raised.

The eggs were fertilised on July 7, and on July 11 some of the larvæ were transferred to Breffit jars with hypertonic water; other larvæ were retained in normal sea-water to serve as controls.

On July 17, when the larvæ had lived for six days in the hypertonic water, a large number of them were transferred to plunger jars. At this time they were showing signs of the division of the cœlomic sacs into anterior and posterior portions (fig. 2).

I had altogether eight plunger jars at my disposal—four being situated on each side of the tank-room. To show how slight are the variations in conditions of light, etc., which make for success or failure, I may mention that only the cultures on one side of the tank-room were successful, and we shall therefore confine our attention to this group of four jars. Of these, one was filled with hypertonic water and the *Nitzschia* would not flourish in it, and as a consequence all the larvæ perished. The remaining three jars were filled with normal sea-water, and each received the contents of a Breffit jar which must have contained over a thousand larvæ; two of the plunger jars received the larvæ from "salted" Breffit jars, and one those from a Breffit jar which had contained normal sea-water. The culture in this jar, therefore, served as a control with which to compare the results of the cultures in the other two. It is necessary to add that all the larvæ which were added to the plunger jars were the offspring of the same cross.

This method of starting a culture of Echinoderm larvæ in a plunger jar differs from the method which I adopted in previous attempts to rear the larvæ of *Echinus esculentus* and *Echinus miliaris.* My former method was to pick out about 300 of the most healthy looking larvæ from a Breffit jar and transfer them to a plunger jar. This method sometimes succeeded, but often failed—in fact one may say that it always failed unless the larvæ were taken from the Breffit jar when they had attained a relatively advanced state of development, when, in fact, all their weaker brethren had perished.

The reason for this seems to be that the eye of the experimenter is unable to select the really healthy larvæ, and that the larvæ must therefore be subjected to a period of struggle—in a word of natural selection—in order that those individuals may be found which are sufficiently vigorous to be able to survive until they complete their metamorphosis.

We may now describe the progress of the cultures in the two plunger jars which contained larvæ which had been subjected to the action of hypertonic sea-water. When the larvæ had attained the age of about 18 days, many of them showed unmistakeable signs of the formation of the rudiment of a right hydrocœle, such as I had observed in the larvæ in my cultures of 1914 and 1915.

But then the development of this rudiment stopped, and it looked as if I were about to obtain a result as indecisive as that which I obtained in 1914 and 1915.

This period was characterised by dull rainy weather which did not favour the growth of the *Nitzschia* in the jars. However, about a week later, sunshine returned, and by means of a persistent addition of *Nitzschia* culture to the jars the growth of the diatom was maintained in a healthy condition, and at last on August 9 larvæ showing an indubitable right hydrocœle were found in the jars. On August 10, 13 and 14 a thorough examination of the contents of the jars was made. The larvæ were taken out in groups of 50, and each group was inspected under the simple microscope. In the beautifully transparent larvæ any trace of a right hydrocœle was at once clearly visible. *In one of the two jars about* 2 *per cent. of the larvæ possessed a right hydrocœle ; in the other, at least* 5 *per cent. showed this structure. In the plunger jar which served as control only one solitary specimen provided with a right hydrocœle was discovered amongst hundreds which were examined.*

At the same time some of the larvæ which had remained in the "salted" Breffit jars developed a right hydrocœle. These larvæ were the offspring of different crosses from the cross the offspring of which was used to form the cultures in the plunger jars.

When one puts together the results of the experiments made during the four years, no reasonable doubt can be left in the mind of anyone that exposure to the action of hypertonic water at an early stage of development really does bring about the formation of a right hydrocœle.

It is probable that by a modification of the methods which I employed, the proportion of larvæ with two hydrocœles could be raised to a much higher figure ; but the difficulty of the experiment lies in this, that to produce a right hydrocœle, or at any rate to produce a fully developed one, two factors are necessary, viz.: (1) the exposure to hypertonic water, and (2) the provision of a superabundance of food. Since the growth of a second hydrocœle carries with it the development of a second set of spines and teeth it is an "expensive" structure and makes large demands on the food supply in the body of the larva.

Since, moreover, the growth of *Nitzschia* varies immensely with conditions, too much or too little light being equally deleterious to it, it is very difficult to ensure the co-operation of this factor in the right strength at the right time.

The appearance of a single larva with two hydrocœles in the control plunger jar serves to warn us that other stimuli besides increased salinity may give rise to this phenomenon, and the circumstance that embryos with two fully developed hydrocœles were observed by Metschnikoff (14) among the specimens which he extracted from the womb of *Amphiura squamata* tends to emphasise this point. Indeed, when we reflect that most cases recorded of larvæ with two hydrocœles have been represented by the occurrence of a single specimen amongst hundreds of normal larvæ, we may feel certain that their development has not been caused by increased salinity.

We may now turn our attention to the structure of the abnormal larvæ which were obtained. In a minority both hydrocœles were equally developed (fig. 8), and in these cases the characteristic pedicellariæ of the right side were absent. The right hydrocœle had induced the formation of a right amniotic cavity, into which its outgrowths projected as a second series of primary tentacles, and from the floor of this cavity pointed spines were produced similar to those belonging to the Echinus-rudiment on the left side. When such larvæ were explored by sections, it was found that on the right side the rudiments of a second Aristotle's lantern were represented by dental pockets just like those on the left side (*d.s.*, fig. 19), and that the right hydrocœle was connected with the right anterior cœlom by a well formed right stone-canal. The right anterior cœlom had been enlarged so as to form a right axial sinus which had become fused with the left axial sinus, and thus there arose a large median cavity lying above the œsophagus; and the madreporic vesicle, which, it will be remembered, normally lies above the œsophagus, was sometimes entirely suppressed (fig. 20), but sometimes persisted as a narrow cavity intervening between the compound axial sinus and the gut (figs. 18, 21).

When describing the development of *Asterina gibbosa* (11), I stated that the madreporic vesicle was a rudiment of the right hydrocœle, because in the abnormal specimens which possessed a fully developed right hydrocœle I was unable to detect a madreporic vesicle. No doubt in these specimens the right hydrocœle had completely suppressed the madreporic vesicle as in the larva represented in fig. 20.

The compound axial sinus may communicate with the exterior by one (fig. 20) or two pore-canals (fig. 21). But in those larvæ in which I found two pore-canals both of them belong to the left side, and the second pore-canal is not therefore to be regarded as an attempt on the part of the right side of the larva to form a structure similar to one normally found on the left side, but rather to a precocious beginning of the process of increasing the number of pore-canals such as occurs in all larvæ at or after the time of metamorphosis.

On the other hand I did discover one larva with a right as well as a left pore-canal (fig. 17). But this larva had been classified by me as a "normal" larva, for in all other respects, as, for instance, in its single hydrocœle and its well developed pedicellariæ, it was entirely normal. The two pore-canals fused with one another in the mid-dorsal line and opened by a median dorsal pore. My results therefore confirm the view of Gemmill that the doubling of the pore-canal and the doubling of the hydrocœle are phenomena which are entirely independent of one another.

In the majority of the abnormal larvæ the right hydrocœle is smaller and less advanced in development than the left one. In such cases one or even both of the pedicellariæ proper to the right side may be present (fig. 10). The right hydrocœle has thus every appearance of being an after-thought, induced by the intervention of an external factor. When it is small it may be entirely devoid of lobes and of an amniotic cavity (fig. 11), and even when an amniotic cavity is developed, a disharmony between it and the hydrocœle is frequently apparent, for one or more of the lobes of the latter, instead of projecting into the amniotic cavity, may project into the blastocœle (*ab.t.*, fig. 10). In some few cases more than one rudiment of a hydrocœle may be formed on the right side; these cases are due to the splitting of the bud which is formed at the hinder end of the right (or left) anterior cœlom. One such specimen was sectioned by me: one of the two right hydrocœles was normally developed, and had associated with it an amniotic cavity; the other was a small structure situated posterior to it, and devoid of an amniotic cavity, but possessing well developed lobes. Another specimen was discovered in which the stone-canal alone was doubled on the left side, a phenomenon obviously to be ascribed to the splitting of the string connecting the hydrocœle bud with the anterior cœlom (*st.c.*, *st.″c.*,″ fig. 19).

B. *The Production of Larvæ Devoid of a Hydrocœle.*

The converse of a larva developing a hydrocœle on both sides would be a larva which developed pedicellariæ on both sides. Such larvæ are very rare: their significance is discussed later. I have, however, discovered a means of producing larvæ devoid both of hydrocœle and of pedicellariæ, but which possess on both sides in the position usually occupied by the hydrocœle a group of spines, and also on both sides a spine or spines in the position usually occupied by one of the pedicellariæ. Such larvæ are represented in figs. 12, 13 and 14, and sections of one are shown in figs. 22 and 23.

In 1915 I possessed a very thick culture of larvæ in a Breffit jar; the larvæ were between six and seven days old, and had had as yet no food, and the well-known signs of degeneration were beginning to appear in many of them. The whole contents of the Breffit jar were then added to a plunger jar in which there was a rich culture of *Nitzschia*. A few weeks later the plunger jar was seen to be filled with a vigorous culture of advanced larvæ, which, to the naked eye, seemed to be completely normal. All had the full complement of eight arms and four ciliated epaulettes. When these larvæ were examined with a lens about one-third of them appeared to be laden with a mass of calcareous growth on both sides. For a brief moment I thought that my hopes of producing a large number of larvæ with a double Echinus-rudiment had been fulfilled; but when these curious larvæ were sectioned no trace of hydrocœle could be discovered on either side of the stomach; nor was there any vestige of a madreporic pore or of a stone-canal. The cœlom had been divided into anterior and posterior portions on each side, but on neither side was the anterior division enlarged so as to form an axial sinus. Dorsal to the œsophagus a median sac, no doubt homologous with the madreporic vesicle, was discovered (*mv.*, fig. 22). Within the loop of the ciliated band (*cil.*, fig. 23), where normally the amniotic invagination is formed on the left side, there was situated on both sides a group of pointed spines (*r.sp.*, *l.sp.*, fig. 23), and dorsal to this loop, in the position usually occupied by the dorsal pedicellaria on the right side, there is situated on both a pointed spine (*r′.sp′.*, *l′.sp′.*, fig. 23).

These spines differ from those present in the normal larvæ in two respects.

(1) If we compare them with the spines normally developed on the right side by the plates which bear the pedicellariæ, then we may notice that whereas the spines in normal larvæ have square ends with diverging points (resembling somewhat in shape old church-towers), the spines in these abnormal larvæ are pointed.

(2) If we compare them with the spines which in the normal larva are borne by the Echinus-rudiment alternating with the primary tentacles, then it is to be noticed that the spines of the normal larva are pointed, but are encircled at their bases by a collar consisting of nervous tissue above and muscular tissue beneath, whereas the spines of these abnormal larvæ are totally devoid of such a collar.

In 1916 and 1917 the experiment which I have just described was repeated and the same result obtained. Amongst all the larvæ which were sectioned one was discovered which possessed a madreporic pore.

DISCUSSION OF THE RESULTS OF THE EXPERIMENTS.

We may say, then, that, as the result of experiments carried on for four years, two points have been determined, viz.: (1) that if the larvæ of *Echinus miliaris* be exposed to the action of hypertonic sea-water for a week commencing with the fourth day of development, many of them will develop on the right side as well as on the left a hydrocœle or water-vascular rudiment, and that in connection with this second hydrocœle all the structures which normally develop in connection with the left hydrocœle may make their appearance, *i.e.*, spines, tentacles and dental sacs; (2) that if the larvæ of *Echinus miliaris* be starved during the first week of their existence, and then placed under favourable conditions both as to food and space, they will continue their development, but many of them will be devoid of both pedicellariæ and hydrocœle, but will have in place of both a group of pointed spines on each side. Such larvæ will, in the majority of cases, be devoid of madreporic pore and axial sinus, but will possess a well-developed madreporic vesicle.

If we confine ourselves for the moment to the first of these results, we cannot help recalling that the result of using hypertonic water on the unfertilised eggs of other Echinoidea is to cause some of them to develop into larvæ without the aid of spermatozoa.

Although Loeb, in his improved method, employed butyric acid to stimulate unfertilised eggs to development, and only used hypertonic water as a subsequent treatment, in order, as he supposed, to correct the cytolytic tendency which had been produced by the acid, yet, in his earlier experiments (10), he found that hypertonic water alone was able to produce parthenogenetic development.

If, however, we make this comparison, another thought is inevitably suggested. Just as the process of development of an unfertilised egg is not something foreign to its nature, which has been forced on it by the hypertonic water, but consists merely in rendering active a latent potentiality, so we cannot assume that the formation of a right hydrocœle is a new accomplishment forced on the larva; it must be merely the rendering actual of a potentiality which exists in the normal larva, but which usually remains in abeyance. That this is so is supported by the occurrence of a single specimen showing the double hydrocœle in a culture reared in normal sea-water which contained hundreds of larvæ.

The most obvious explanation of this latent potentiality is to assume that the Echinoderms are descended from some type of animal which had a hydrocœle-like structure on each side. Such a hypothetical ancestor is very nearly realised in the existing genus *Cephalodiscus*, which belongs to the group of the Hemichorda, and is thus related to the vertebrate phylum.

Cephalodiscus possesses two groups of long ciliated tentacles, and each group spring from a semicircular canal situated at one side of the mouth. Gilchrist (8) has shown that the living *Cephalodiscus* crawls about on its præoral lobe, and occasionally fixes itself to the substratum by the surface of this lobe, exactly as I have shown that the larva of *Asterina gibbosa* does (11). The theory that Asteroidea, and inferentially all other Echinodermata, are descended from an ancestor like *Cephalodiscus*, was fully worked out by me in my paper on the development of *Asterina gibbosa*, and the results of the experiments recorded in this paper confirm my confidence in the soundness of that theory.

But, when I described the first two larvæ of *Echinus* with two hydrocœles which came into my hands (14), I pointed out that the probable descent of Echinodermata from a *Cephalodiscus*-like ancestor affords at best only a partial explanation of the phenomena with which we are concerned For it is to be noted that spines provided with a nervous and muscular collar are characteristic of the highly developed Echinoidea, and are not found in the more primitive Asteroidea, and the same is true of the highly developed teeth and jaws which constitute Aristotle's lantern. It is certain that the bilaterally symmetrical ancestor possessed none of these structures; but that they were evolved long after the preponderance of growth of the left hydrocœle over the right one had led to the conversion of the bilateral symmetry into a radial symmetry. The theory that there was a bilateral ancestor of Echinodermata therefore cannot account for the existence of a double set of highly developed spines and dental sacs in the abnormal larva.

In the paper on these two abnormal larvæ, I suggested that the conversion of a portion of the ectoderm of the left side into highly developed spines, and the development of dental pockets from the wall of the left posterior cœlom, were due not to the inborn capacities of these tissues, but to the acquisition of powers conferred on them by some chemical substance, in a word, some **hormone,** which emanated from the hydrocœle bud. I said, further, that where a similar bud was formed on the right side, it emitted a similar substance, which forced the right ectoderm and the wall of the right posterior cœlom to undergo corresponding changes, although they had never, in the history of the race, been modified in this manner. I assumed that the hydrocœle bud was the prime mover in the wonderful array of changes which led to the formation of an Echinus-rudiment, because in normal development it is the first part of this rudiment to appear, but this assumption is proved to be justified by the results of the experiments recorded in the present paper. For we have learned that when the right hydrocœle is small it may develop lobes, but may have no power to cause the formation of an amniotic invagination (fig. 11), and therefore no spines are formed, and the wall of the cœlom is also unaffected and no dental pockets are developed. We can, therefore, have a right hydrocœle without a right amniotic invagination and right dental sacs, but we cannot have these latter structures without a right hydrocœle.

I possess one larva which has a right hydrocœle developing long lobes and a vestigial amniotic invagination which is entirely devoid of relation to these lobes.

The fact that a right hydrocœle can co-exist with one or more pedicellariæ raises some interesting questions. These pedicellariæ are situated externally to the loop of the ciliated band within which the amniotic invagination is formed, so that when they are not formed, this non-appearance cannot be due to the right hydrocœle taking up the space which is normally occupied by them. It seems far more probable that just as a hormone emanating from the hydrocœle causes the formation of a right amniotic cavity, so another hormone derived from the same source tends to inhibit the formation of pedicellariæ. When pedicellariæ are nevertheless developed on the same side of the larva, this seems to be due to the arrested growth of the hydrocœle bud, so that when it really does begin to grow, the normal development of the right side has proceeded to such lengths as to be irrevocable, *for in every larva which has pedicellariæ on both sides a hydrocœle is also present on both sides although one of them is usually vestigial.*

In 1917, owing to a temporary arrest of the growth of the *Nitzschia* in the plunger jars, the development of the right hydrocœle stood still for about 10 days and in the meantime the ominous knobs which are the first indications of the formation of the pedicellariæ made their appearance on the right side of the larvæ, and I feared that all chance of the formation of a well-developed right hydrocœle was lost. But to my intense delight this fear proved groundless, for when a more abundant food supply was available the right hydrocœle resumed its growth in spite of the fact that by that time one or even two pedicellariæ had been formed. But the occurrence of larvæ in which there were present a hydrocœle and two pedicellariæ on each side enables us to go further in our analysis. Such a larva can be explained only if we assume that the influences emanating from a hydrocœle not only tend to inhibit the formation of pedicellariæ on the same side but to determine their formation on the opposite side of the larva. If we assume that in these larvæ the growth of both hydrocœles had been arrested at an early stage, but after the stage at which the stimulus to form pedicellariæ on the opposite side had already gone forth from them, and that then, after the formation of these organs on both sides had been determined, further nourishment became available and the left hydrocœle developed further, the structure of such larvæ can be explained.

The second main result obtained by my experiments concerns the further development of larvæ which have been starved during the first week of their existence. Such larvæ have no hydrocœle—it is probable that that part of the cœlomic rudiment which should have developed into the hydrocœle becomes absorbed during the period of hunger. In the further history of these starved larvæ we see the potentialities of development of the *Echinus* larva when the hydrocœle bud has been removed. It is, therefore, most instructive to note that the power of forming pedicellariæ has likewise disappeared, and this result confirms the conclusion at which we had already arrived, viz., that the power which determines the formation of pedicellariæ emanates from the hydrocœle.

How are the extraordinary potentialities of the right side to be explained, potentialities, it may be remarked, which are never called into activity during the normal life of the species, and so far as we can judge have never been utilized in the past history of the race? We seem shut up to some such hypothesis as this. When the *Cephalodiscus*-like ancestor still possessed two hydrocœles, and whilst it was developing into a Proto-echinoderm with one hydrocœle its dominant activities were comprised in the writhing grasping movements of the tentacles which were the outgrowths of these hydrocœles. These activities caused modifications to take place in the neighbouring tissues; and if one believes, as I do, in the inheritability of acquired characters, one may assume that the tendency to produce these modifications was inherited, so that the modifications were eventually evoked by the mere presence of the hydrocœle bud before the activities of its lobes had begun. We must further assume that the hormones which produced the modifications, tended to be stored in the hydrocœle bud itself—and that both hydrocœles were ultimately derived from the same small area of the cœlomic wall. All the hormones therefore necessary to produce the modifications which were acquired *after* the *Cephalodiscus*-ancestor by the loss of one hydrocœle had become an Echinoderm, were still situated in the same place; and so, when this area under the influence of stimulation produced two hydrocœles instead of one, these hormones were shed abroad on both sides of the larva, although they had only been evolved in connection with the left side.

This hypothesis may help us a little way, but no one is more conscious than I am how incomplete and unsatisfactory it is. We ask in vain what sort of chemical composition we must ascribe to the right ectoderm and to the right cœlomic wall, so that they are able under the influence of a hydrocœle bud to give rise to structures to which in the history of the race they have never given rise. It is well to remind ourselves that this amazing phenomenon does not stand alone. The facts that when the eye-stalk of a shrimp, including the underlying optical ganglion, is cut out, the stump regenerates not an eye but an antenna, and that further when the primary optic vesicle of a young tadpole is cut off from the brain and pushed backwards under the skin into the region of the shoulder it will force the local ectoderm to form for it a lens, warn us that living developing tissues do not obey the laws with which we become familiar when we study dead matter.

In many cases, at any rate, the body of an embryo is not, like a picture puzzle, a mosaic of pieces each destined to form a particular organ, but consists of sheets of indifferent material "without form and void" on which a formative "something" works and evokes the beautiful detail of the adult structure. As Driesch (3) has expressed it, "Ein jedes jedes kann."

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EXPLANATION OF PLATES.

LIST OF ABBREVIATIONS.

ab.t., aberrant tentacle, *i.e.*, lobe of the right hydrocele which projects into the blastocœle and not into the amniotic cavity; *a.l.*, antero-lateral ciliated arm; *am.*, amniotic cavity; *an.*, anus; *ax.*, axial sinus; *ax'.*, axial sinus developed on the right side from the right anterior cœlom; *calc.*, calcareous plate; *cil.*, portions of the ciliated band of the larva forming the loop within which the amniotic invagination is formed both on the right and on the left side; *cœ.*, cœlom; *d.a.*, dorsal arch, *i.e.*, the calcareous rod lying above the œsophagus in the larva; *d.c.ep.*, dorsal ciliated epaulette; *d.s.*, dental sac associated with the left hydrocœle; *d.s'.*, dental sac associated with the right hydrocœle; *Ech.*, Echinus-rudiment; *int.*, intestine; *l.a.c.*, left anterior cœlom; *l.hy.*, left hydrocœle; *l.p.c.*, left posterior cœlom; *l.sp.*, left group of spines within the ciliated loop; *l'.sp'.*, left spine outside the ciliated loop; *mad.*, madreporite; *m.p.*, madreporic pore; *m'.p'.*, additional madreporic pore; *m.sp.*, madreporic spine; *œs.*, œsophagus; *p.c.*, pore canal; *p'.c'.*, additional pore canal; *p.d.*, postero-dorsal ciliated arm; *ped.*, pedicellaria; *p.o.*, post-oral ciliated arm; *pr.o.*, præ-oral ciliated arm; *r.a.c.*, right anterior cœlom; *r.hy.*, right hydrocœle; *r.p.c.*, right posterior cœlom; *r.sp.*, right group of spines within the ciliated loop; *r'.sp'.*, right spine outside the ciliated loop; *sept.*, remains of the septum which formerly divided right and left axial sinuses from one another; *st.c.*, stone-canal; *st.'c'.*, additional stone-canal on the right side; *st''.c''.*, additional stone-canal on the left side; *stom.*, stomach; *v.c.ep.*, ventral ciliated epaulette.

N.B.—Skeletal structures are indicated in the drawings of whole larvæ by heavy black lines.

PLATE 4.

Fig. 1.—Young larva between three and four days old viewed from the ventral aspect. Preserved in osmic acid, followed by Müller's fluid, which has dissolved the skeleton. Magnification 330 diameters. Note the stomodæum just about to open into the œsophagus (*œs.*). Only the post-oral ciliated arms are developed (*p.o.*). This larva is approximately at the stage of development at which larvæ were transferred to hypertonic water in 1914, 1915 and 1916, and a little younger than that at which they were transferred to this water in 1917.

Fig. 2.—Larva 12 days old viewed from the dorsal aspect, showing on the left side the formation of the hydrocœle and on the right side the almost completed separation of the cœlomic sac into anterior and posterior portions.

Drawn from life from the cultures of 1914. Magnification 90 diameters. This larva is about the stage of development at which, in 1917, larvæ were transferred from hypertonic water to normal water in the plunger jar.

Fig. 3.—Larva 18 days old viewed from the dorsal aspect, showing the first appearance of a rudiment of the right hydrocœle. This larva was transferred to hypertonic water on the third day and remained in it. Drawn from life from the cultures of 1914. Magnification 80 diameters.

Fig. 4.—Larva about 32 days old, from the cultures of 1917, reared entirely in normal water, and viewed from the dorsal aspect. Preservation 40-per-cent. formalin. Magnification 80 diameters. This larva and that represented in the two following figures give an idea of the stage of development attained by the larvæ in the control plunger-jar when the experiments of 1917 were closed; *m.p.*, madreporic pore; *st.c.*, stone-canal, the reference line points to the opening of this canal into the axial sinus (*ax.*).

PLATE 5.

Fig. 5.—Larva entirely similar to that represented in fig. 4, viewed from the left side; *mad.*, madreporic plate; *Ech.*, Echinus-rudiment.

Fig. 6.—The same larva as that represented in fig. 5, viewed from the right side; *calc.*, calcareous plate within the loop of the ciliated band midway between the two pedicellariæ.

Fig. 7.—Larva 40 days old, which had been exposed to hypertonic sea-water, viewed from the dorsal aspect. Drawn from life from the cultures of 1916. Magnification about 50 diameters.

Note *l.a.c.*, the left anterior cœlom, *i.e.*, the axial sinus into which the stone-canal (*st.c.*) opens; *r.a.c.*, the right anterior cœlom, expanded into a supplementary axial sinus; *r.hy.*, the right hydrocœle.

Figs. 8–11, inclusive, represent larvæ about 32 days old, from the cultures of 1917, which have been exposed to the action of hypertonic water and have been subsequently transferred to normal sea-water in a plunger jar. All have been preserved in 40-per-cent. formalin, and all are viewed from the dorsal aspect under a magnification of 65 diameters.

Fig. 8.—Larva with two hydrocœles of almost equal size and no pedicellariæ.

Note *ab.t.*, aberrant tentacle, a lobe of the right hydrocœle projecting into the blastocœle; *m.p.*, *m'.p'.*, the two madreporic pores.

Fig. 9.—Larva with the right hydrocœle smaller than the left but devoid of pedicellariæ.

Note *a.x.*, the single axial sinus into which the two stone-canals (*st.c.*, *st'.c'.*) open; *m.p.*, the single madreporic pore.

PLATE 6.

Fig. 10.—Larva with two hydrocœles of nearly equal size but with a single pedicellaria on the right side (*ped.*); *m.p.*, the single madreporic pore; *v.c.ep.*, the ventral ciliated epaulette seen through the transparent body-wall.

Fig. 11.—Larva with two hydrocœles, of which the right is small and without lobes. No amniotic cavity is developed on the right side; *ax.*, left axial sinus; *ax.'*, right axial sinus; *m.p.*, *m'.p'.*, the two madreporic pores. Two pedicellariæ are developed (*ped.*).

Figs. 12–14 represent larvæ from the cultures of 1917 which had been starved for the first week and then generously fed. They were about 32 days old, and were preserved in 40-per-cent. formalin. Magnification 65 diameters.

Fig. 12.—Larva viewed from the dorsal aspect: *l.sp.*, the left group of spines within the loop of the ciliated band; *r.sp.*, the right group of spines within the loop of the ciliated band; *l′.sp′.*, spine on the left side outside the loop of the ciliated band; *r′.sp′.*, spine on the right side outside the loop of the ciliated band.

Fig. 13.—Larva viewed from the left side: *m.sp.*, madreporic spine.

Fig. 14.—The same larva as that shown in fig. 13, viewed from the right side.

Figs. 15–23 represent transverse sections through larvæ preserved in Bouin's fluid. All are from the cultures of 1917, and are about 32 days old, except where otherwise stated.

PLATE 7.

Fig. 15.—Section of the normal larva showing the mutual relations of axial sinus, madreporic vesicle, pore-canal and madreporic pore. Magnification 120 diameters.

Fig. 16.—Section of normal larva to show the mutual relations of the Echinus-rudiment and the pedicellariæ. Magnification 120 diameters.

Fig. 17.—Section of larva with right and left pore-canals (*p.c.* and *p′.c′.*). In another section from the same series these canals are seen to unite, to open by a single median pore to the exterior. Magnification 105 diameters.

Figs. 18 and 19 represent two sections of the same larva. The larva possessed two well-developed hydrocœles and one pedicellaria. Magnification 105 diameters.

PLATE 8.

Fig. 18 shows the single pore-canal, the single median axial sinus (representing the combination of right and left axial sinuses), and the relation of this compound axial sinus to the madreporic vesicle.

Fig. 19 shows the two hydrocœles: *d.s.*, dental sac of the left side; *d′.s′.*, dental sac of the right side.

PLATE 9.

Fig. 20.—Section of a larva with two hydrocœles and one pore-canal. The median axial sinus has caused the complete suppression of the madreporic vesicle. Magnification 120 diameters.

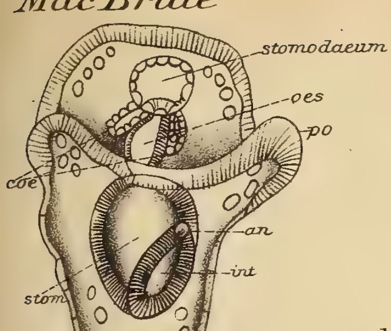
Fig. 21.—Section of a larva 40 days old, from the cultures of 1916, which has two hydrocœles. Two madreporic pores (*m.p.* and *m′.p′.*) are present, but both belong to the left side. Magnification 120 diameters.

Figs. 22 and 23 represent two sections of the same larva. This larva belongs to the cultures of 1917, and is one of those which was starved and then generously fed. Magnification 120 diameters.

PLATE 10.

Fig. 22 shows the median madreporic vesicle (*m̀.v.*).

Fig. 23 shows the groups of spines which replace the Echinus-rudiment and the pedicellariæ.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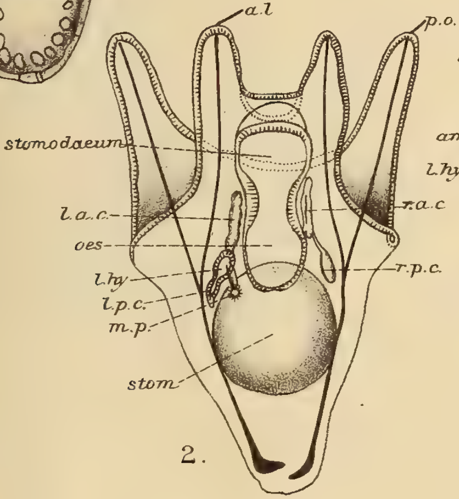
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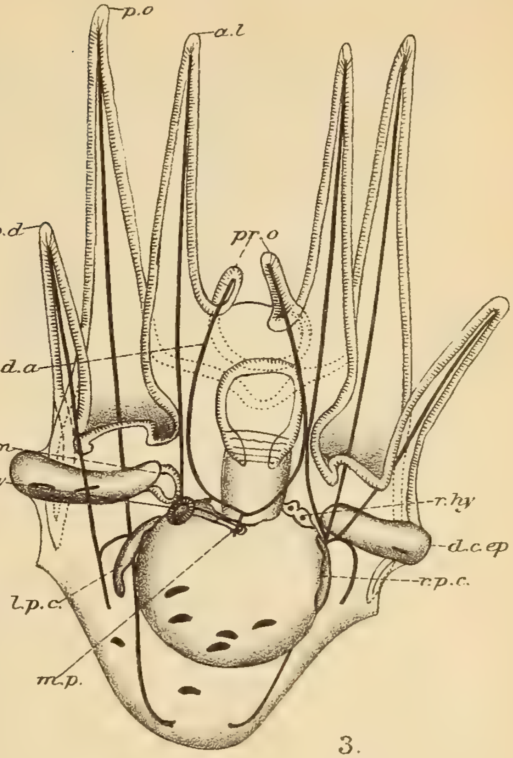
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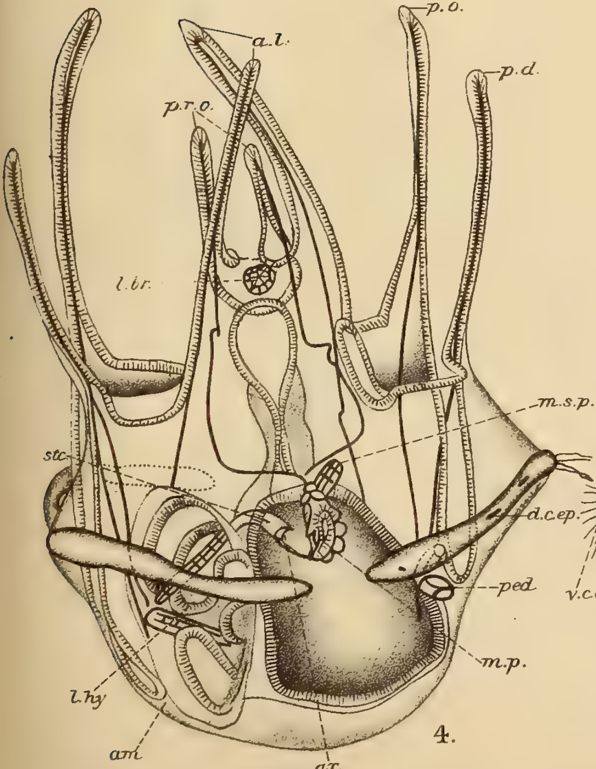
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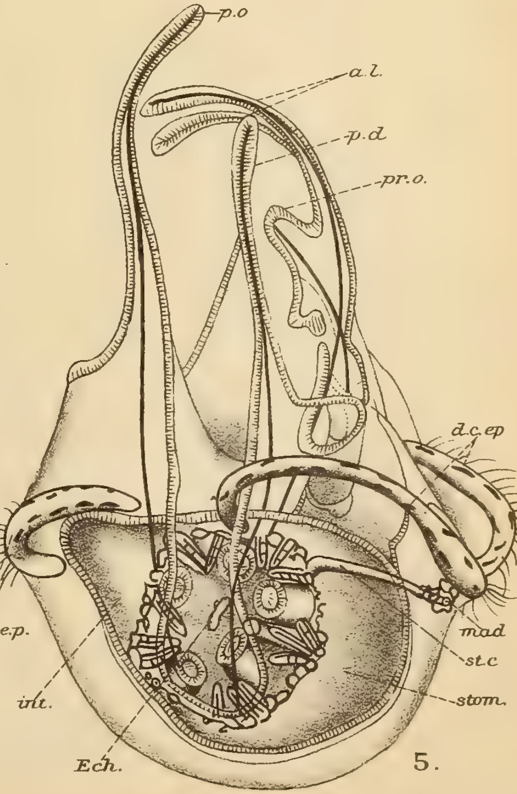
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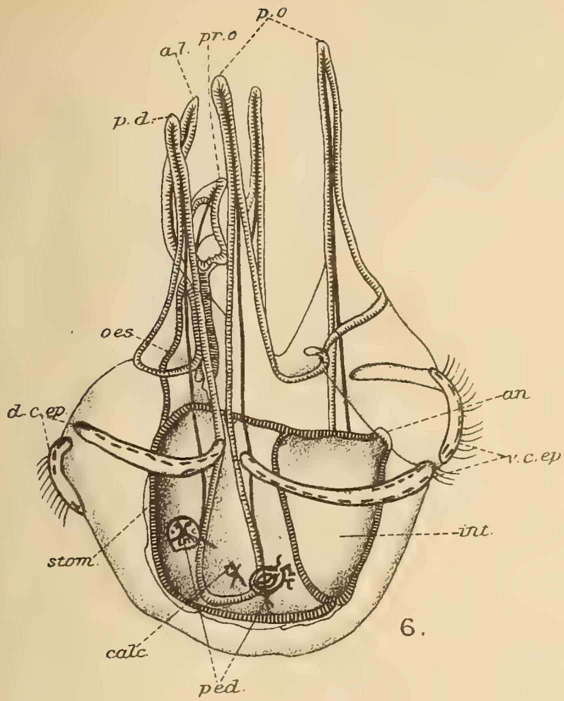
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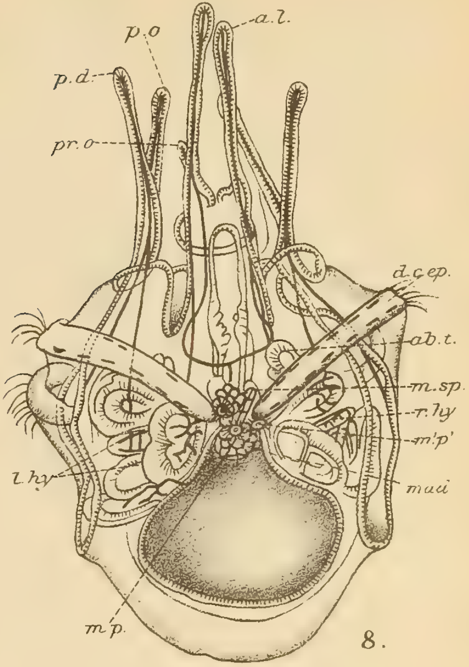
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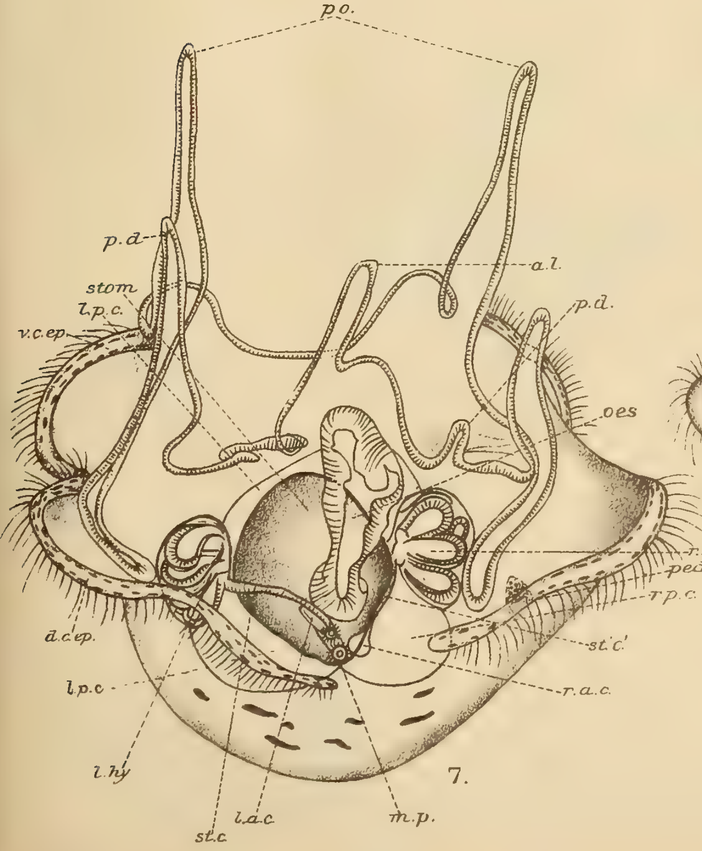
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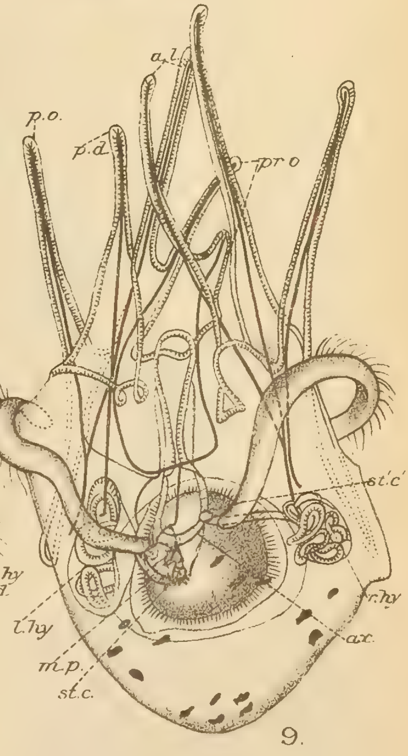
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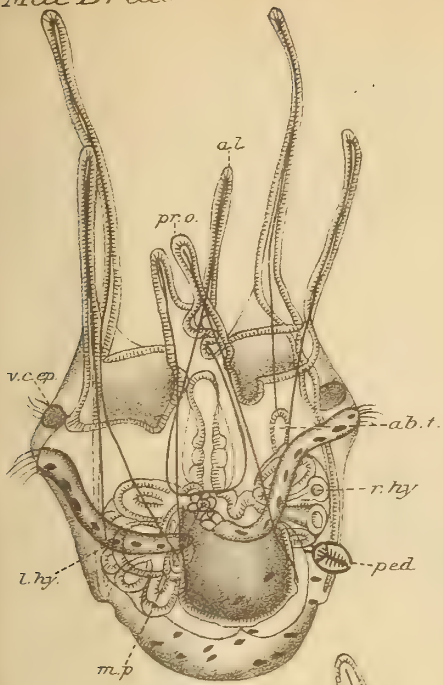
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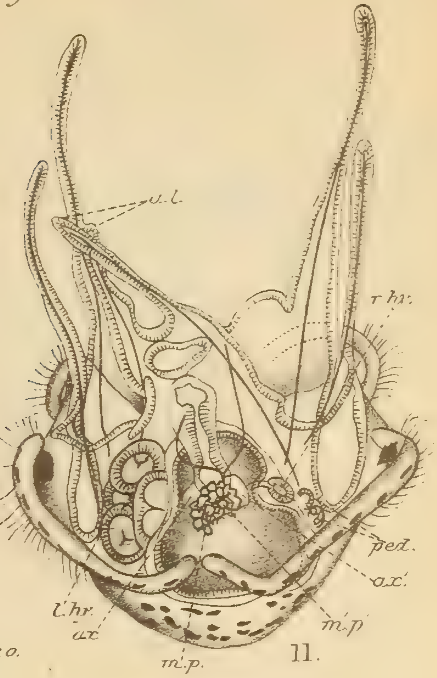
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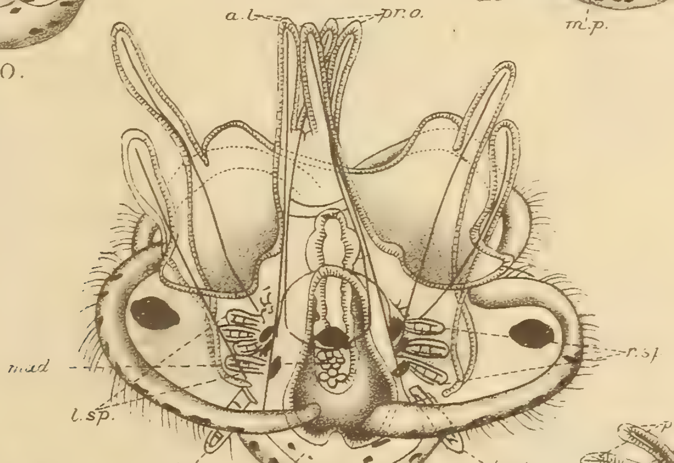
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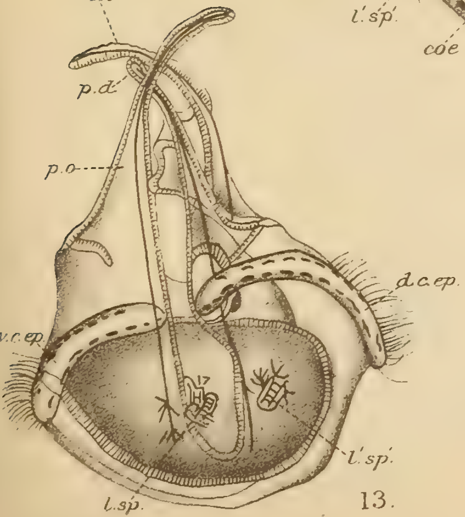
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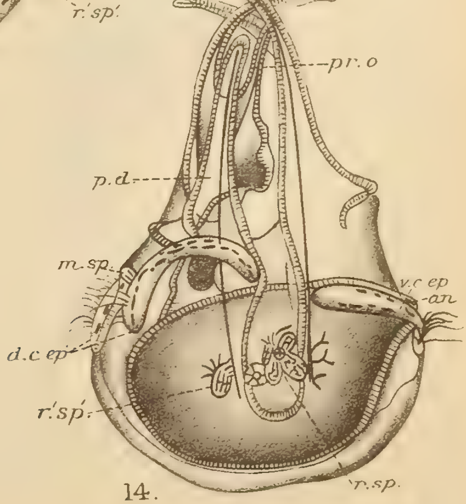
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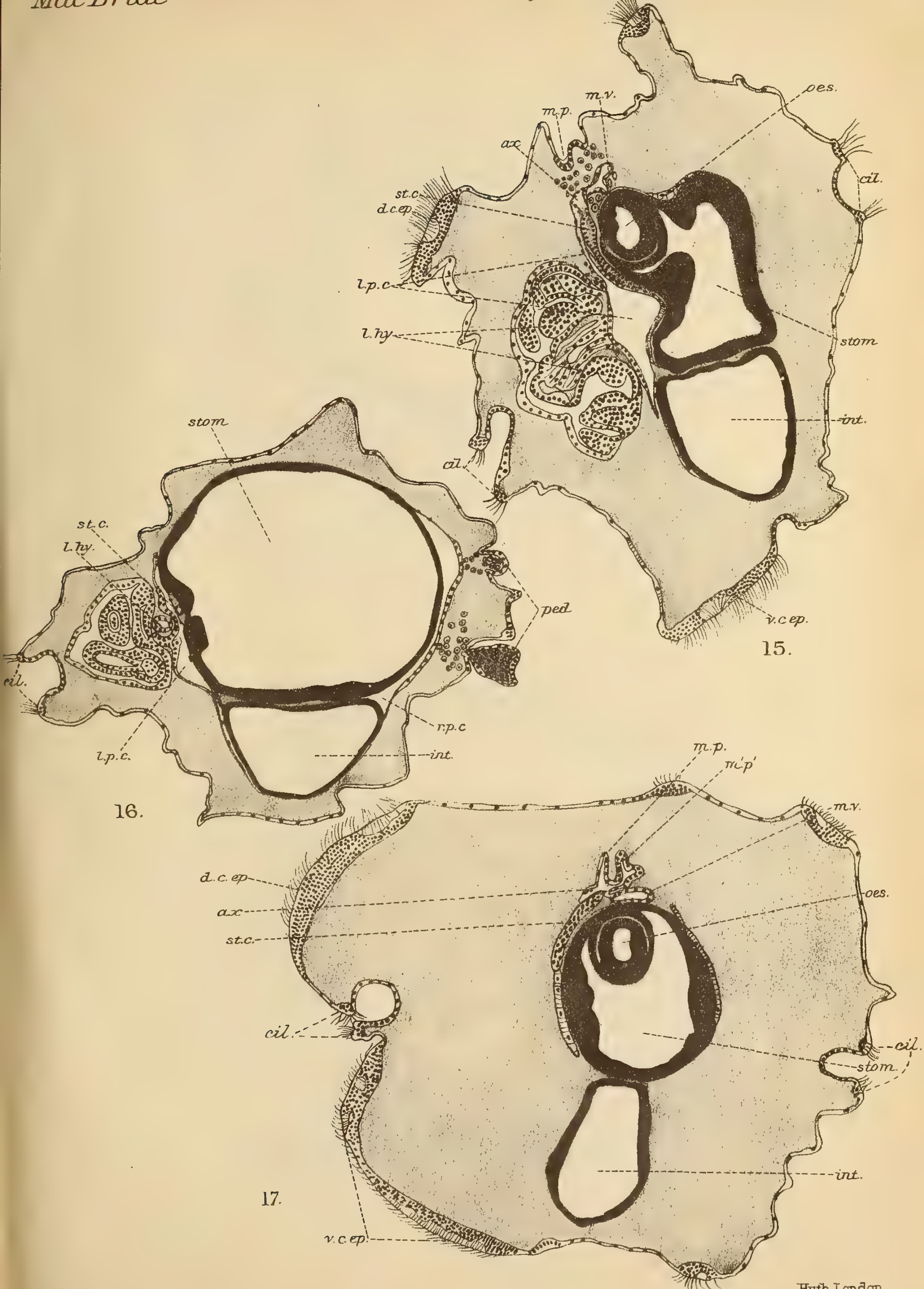
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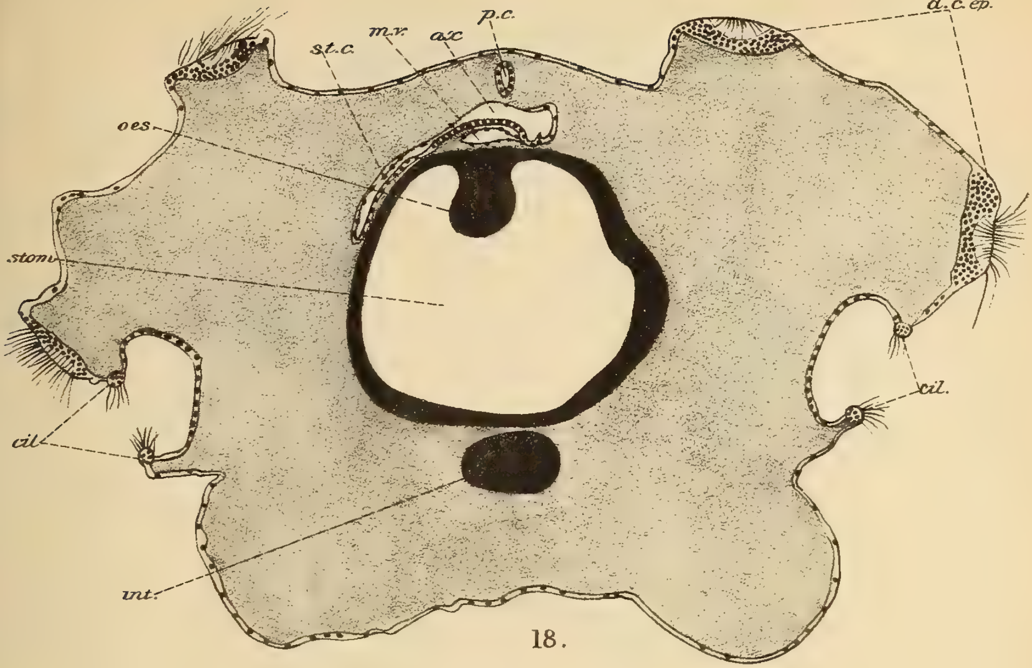
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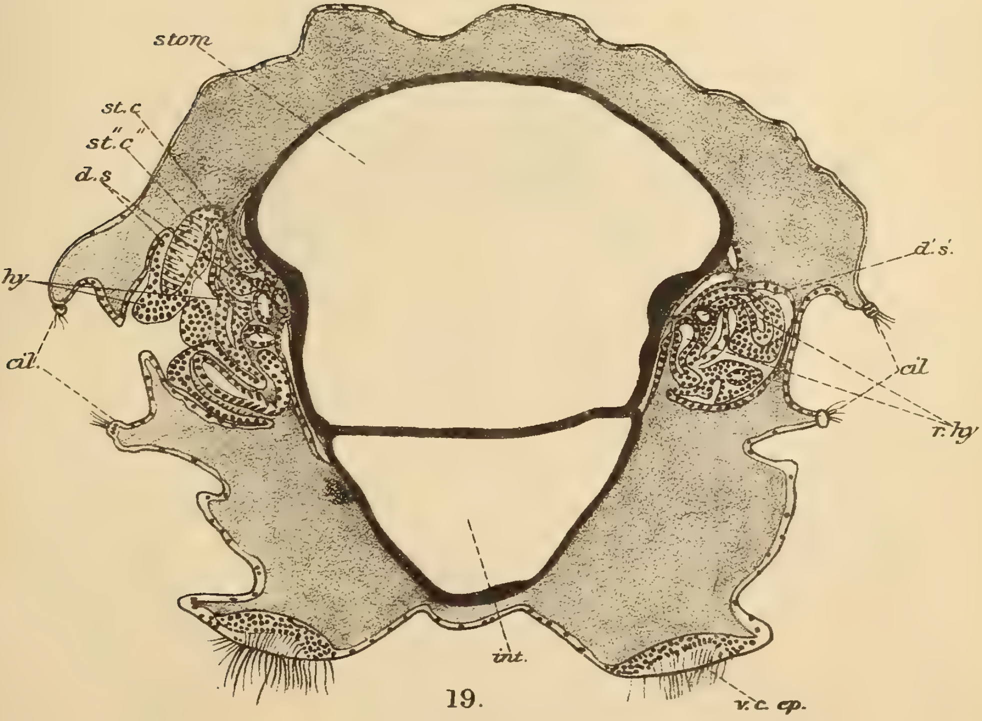
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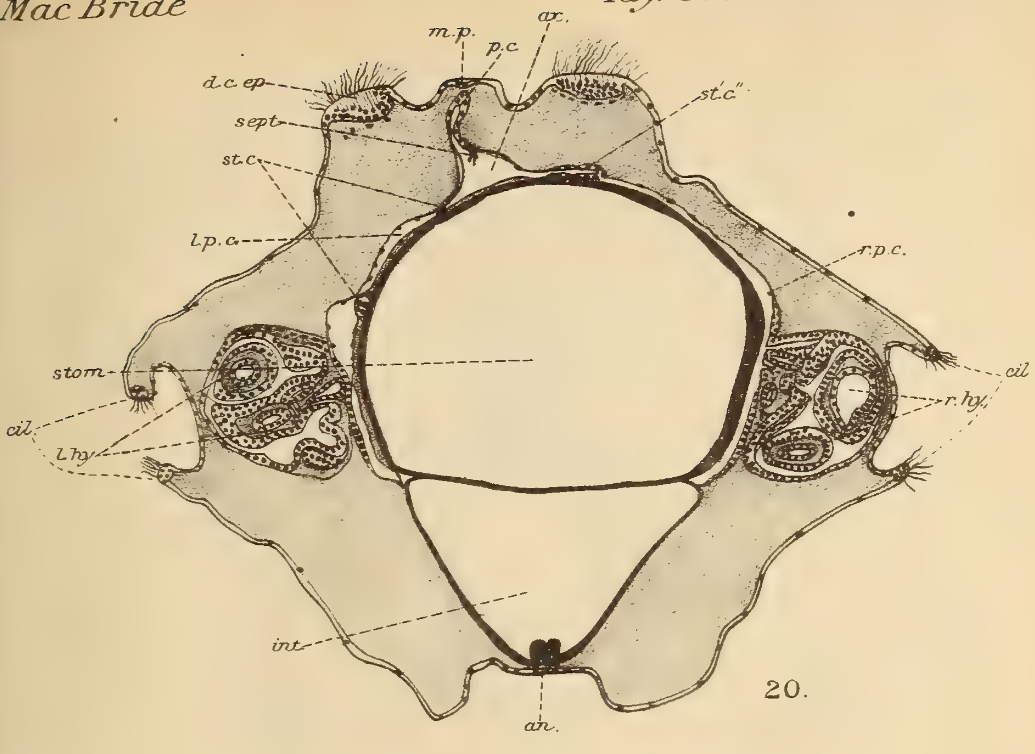
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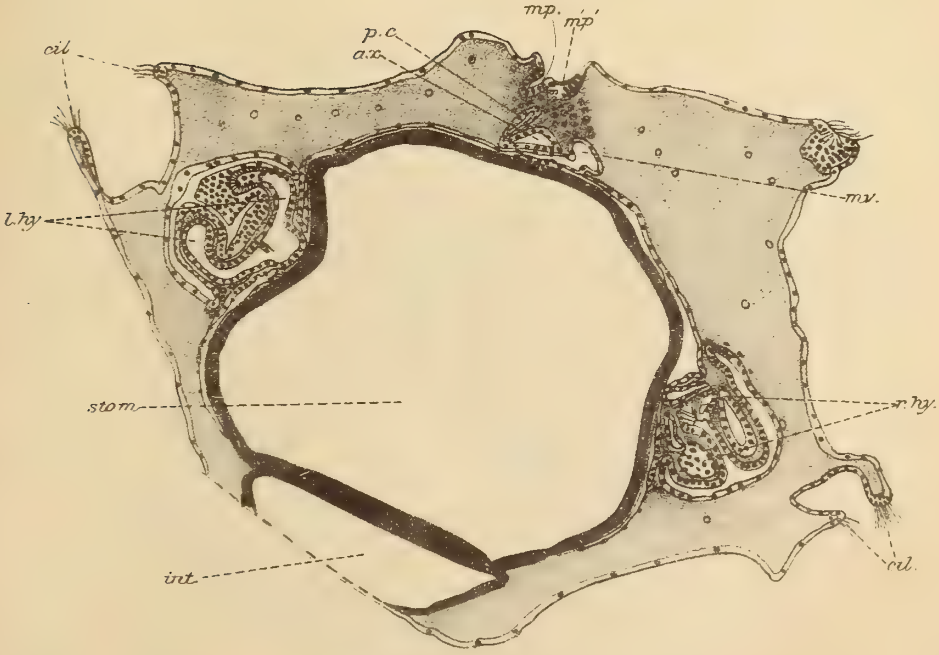
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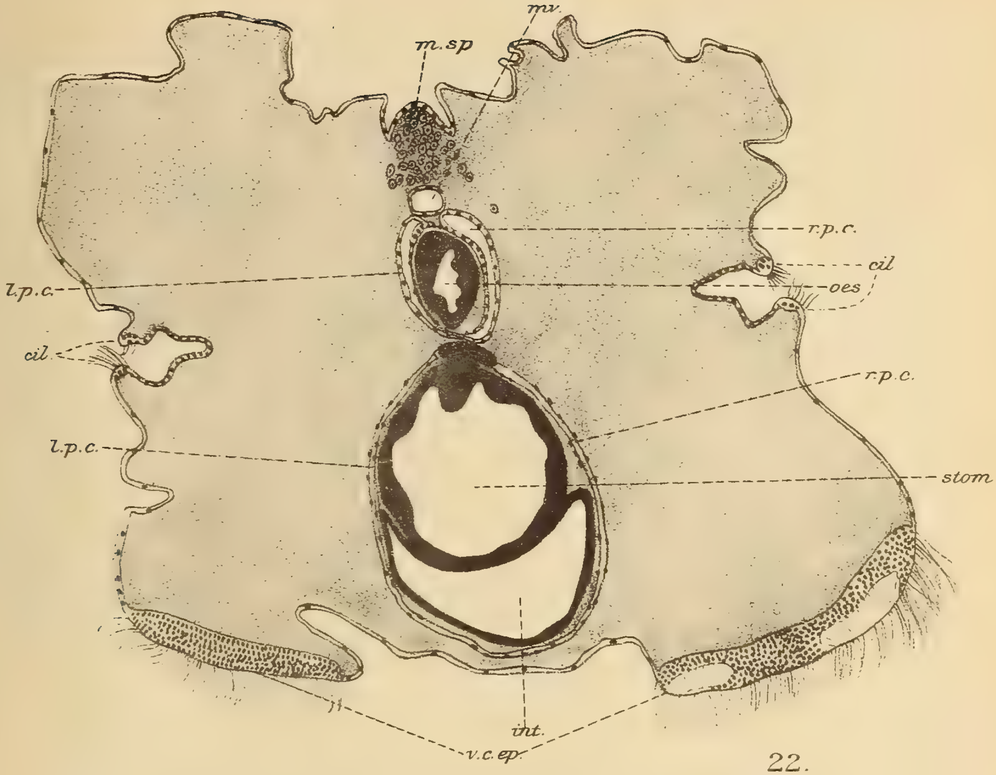
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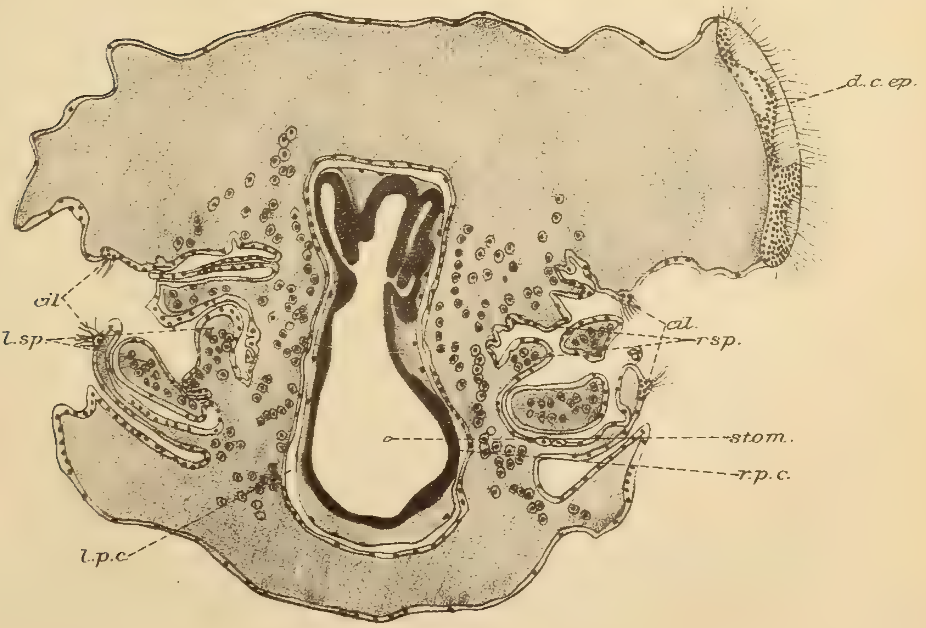
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Huth London

Changes of Electrical Conductivity under Geotropic Stimulation.

By James Small, M.Sc. (Lond.), F.L.S.

(Communicated by Dr. A. D. Waller, F.R.S. Received May 11, 1917.)

The very short perception period* for the epicotyl and hypocotyl of various plants, which Fitting (3) has proved, and the presence of geotropic response in the absence of starch grains in many fungi and higher plants tend to indicate that the starch grain or statolith apparatus is not absolutely necessary for the perception of gravity by plants. The differential effect of gravity on the permeability of the upper and under sides of the pulvinus of *Phaseolus* indicates the possibility of a similar effect being produced in the roots of the plants.

Fitting's numerous experiments were examined in order to ascertain whether the strength of the geotropic reaction bore any constant relation to the geotropic stimulus. Putting the data given in Fitting's Table 10 [(3), Teil I, p. 282] in the form of a graph, we get a curve (fig. 1) which is

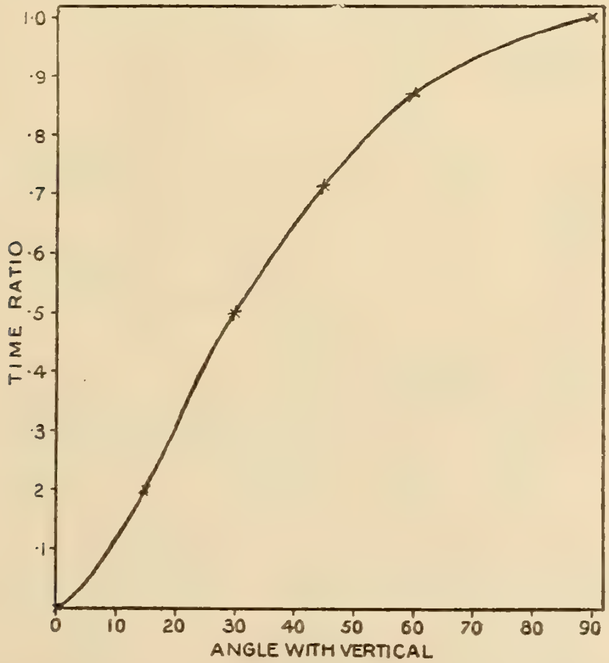

Fig. 1.

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* *Perception period* is used here, not in the psychological sense, but in the sense generally accepted among botanists, and defined as the smallest fraction of the *presentation time* which gives a cumulative effect when the stimulus is repeated for a sufficient number of times with less than the *relaxation time* between the successive stimulations.

logarithmic in the centre and shows divergences at the extremities, the ordinates being the ratios of the times of exposure at 90° to the times at other angles to the vertical, and the abscissæ being the angles with the vertical. In this way we get the strength of the reaction, which varies directly as the time of exposure, plotted against the strength of the stimulus, which varies directly as the angle with the vertical. The resulting graph is the typical sigmoid curve obtained by Waller (9) for the response by animals to various stimuli. This more or less logarithmic relation is also proved by the fifty-eight experiments on the perception of minimal angle differences by the epicotyls of *Vicia Faba* and *Phaseolus multiflorus* and by the hypocotyls of *Helianthus annuus*, which Fitting (3, Teil I, pp. 306–310) has published (fig. 2).

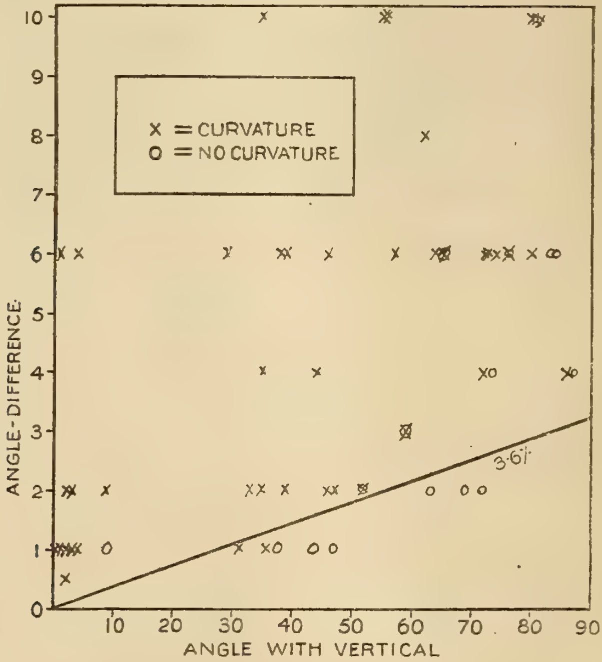

Fig. 2.

From these published results of other investigators the hypothesis was formed that the proximate mechanism of geotropic response in plants is a change in the permeability of the protoplasm in the cells of the perceptive region. Such changes involve changes in the electrical resistance of the tissue [*cp.* McClendon (5) and Osterhout (6*b*)].

EXPERIMENTAL PROOFS.

Method.

The hypothesis was tested by some preliminary experiments with the Kohlrausch bridge, and it was found that the turning of the root horizontal always caused an immediate decrement of the resistance, which was interpreted, as in McClendon's experiments (5), as increase in permeability to ions. This decrement of resistance was distinctly less in the upper side of the root than in the lower. A more accurate apparatus, which will now be described, was then set up, and the results are given below.

The Kohlrausch modification of the Wheatstone bridge consists in (1) the use of an alternating current which prevents local electrolysis in the tissue; (2) the use of a telephone by which the position of the sliding contact is regulated so that the least amount of sound is heard in the telephone. This "silence" point or position of minimum sound is the point at which the resistances have the well known relationship to one another. Fig. 3 shows the arrangement of the apparatus diagrammatically, AB being the metre bridge, C the sliding contact, D the telephone, E the induction coil, F a single accumulator, G a plug key for putting the accumulator into or out of action, H a tapping key, R a resistance box, and P the plant or unknown resistance. By means of the tapping key the injurious effects of the passage of the current through the tissue are minimised. When the silence point is obtained the position of C is read off and the resistance calculated from the ratio AC : CB : : P : R.

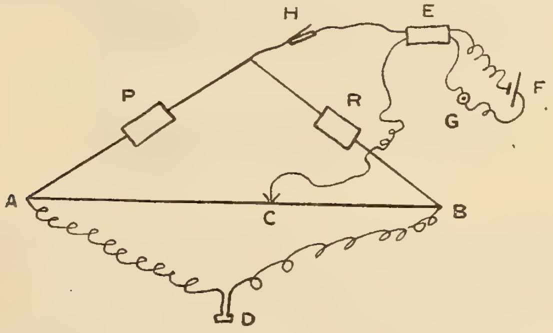

FIG. 3.

The resistance to be determined was that of the tissue at one side of the root-tip of *Vicia Faba* from 1 mm. to 2 mm. from the apex. The ends of two fine platinum wires were bent at right angles, the bent ends being 0·75 mm. to 1·5 mm. in length. About 1·5 cm. of the root was covered with paraffin wax melted at a temperature of 45° C. As the root was dipped into the wax and quickly withdrawn, no injurious effects were observed. This was tested by removing the wax tip and growing the beans for about 10 days afterwards. The wires were inserted on the same side of the root, the first 1 mm. from the apex and the second 2 mm. from the apex. This operation requires much practice and a considerable amount of patience if the root is not to be injured to such a degree that the succeeding stages are useless. The wax covering serves the double purpose of keeping the root from drying and of insulating the wires from the surface of the root. A root-tip covered with wax in this way will remain fresh for hours after the unwaxed portion is strongly withered, and the aërating system of the root of *Vicia Faba* is efficient enough to provide the necessary opportunities for respiration in the covered portion of the root (4). The wires were fixed in position by a plug of wax melted around them, so that a longitudinal section of the root-tip, fitted with wires, would show something like fig. 4. The placing of the plant at any desired angle was accomplished by means of the apparatus figured (fig. 5).

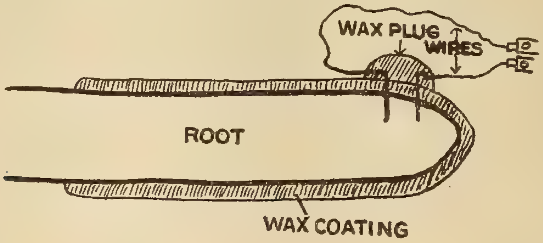

Fig. 4.

The supports C and E were clamped to a shelf, AB. A wooden support, D, with a socket in it, was supported by C. The angle was indicated upon a scale, O, marked upon a smooth piece of mahogany with a socket at the centre of the semicircle. The scale was divided into 36 angles of 5° each, and holes were made in the proper places so that a plug, P, could be inserted to support the swinging portion of the apparatus at any required angle. The orientation of this scale was checked by means of a plumb line T. For convenience in fixing the plant a piece of sheet cork, H, was screwed to a wooden base, G. This base had a vertical line drawn along the centre of the length and another line at right angles to it, at about one-third of the length from the top. At the ends of the latter line steel rods, G′, G′, were inserted to give an axis upon which the part swung when the free ends were inserted in the sockets of D and O. The sheet cork was thoroughly waxed, and a thread, S, was fixed along it coinciding with the vertical line on the wooden base. The orientation was checked with a set square, a plumb line, R, and the thread, which was waxed over for insulation. Two blocks of paraffin wax, K, K, were fixed to the cork near the base, and to each a brass binding screw, I′, was fixed by means of wire staples, which were sunk in the wax, but did not penetrate to the cork. Two terminals, L, L, consisting of thin platinum wires fixed to small strips of zinc, were then attached to the binding screws. Connection with the induction coil and the metre bridge was made with insulated wires fixed in the binding screws. To the side of G was fixed a large pin bent so that it formed an indicator on the scale, O, and by resting on the plug, P, kept the whole in position. The bean, after the root-tip had been coated with wax, was fixed with two pins to the sheet cork, so that the root was parallel to the surface of the cork and to the vertical thread, S. The wires were then inserted on the "front" of the root, M, with all due precautions. After the plug of wax had been placed in position to keep the wires from moving, the unwaxed portion of the plant was covered with wet lint to keep it moist.

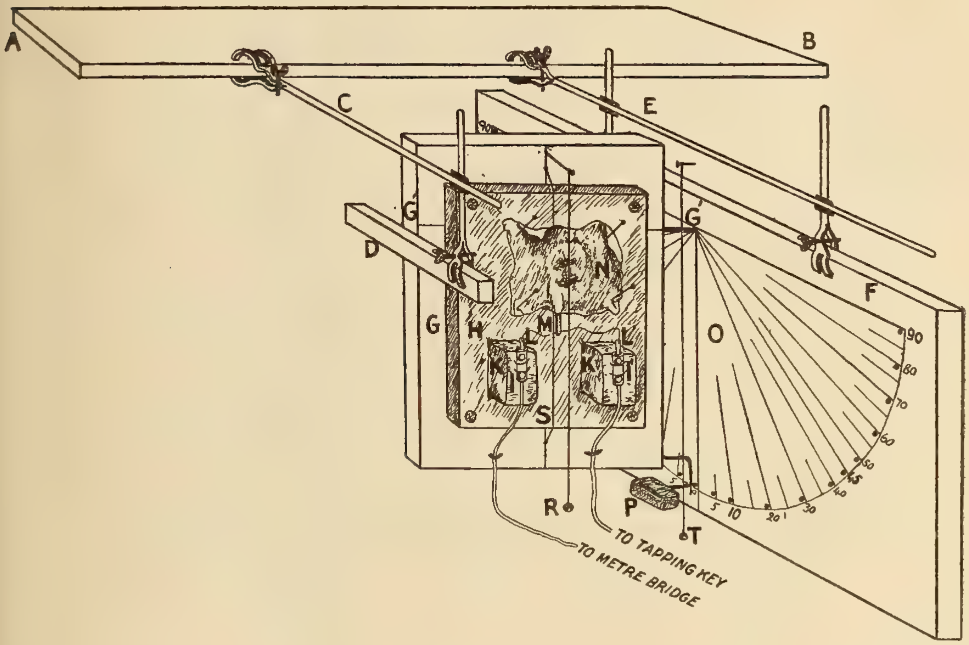

FIG. 5.

By tilting G and fixing it in position by means of the indicator pin and the plug P, the root could be placed so that the wires were in either the upper or the lower side. In the records of the experiments given below + angles were taken as those where the wires were in the physically upper side of the root and − angles as those in which they were in the physically lower side.

The scale O acted as a light screen, so that the root was not subject to any heliotropic stimulus.

The Wound Reaction.

It may be supposed that two wires cannot be inserted into the root-tip without inducing some sort of a wound reaction, and the passage of even an alternating current is almost sure to have some effect on such a delicate structure. The first experiment was, therefore, to determine the resistance at given intervals of time with the root in the vertical position. Preliminary experiments had shown that the effect of the current could be eliminated by taking the readings within a minute and allowing 15 mins. between each. Fifteen-minute readings, therefore, were taken with the root vertical, and the results show that during the first 30—40 mins. there is an injury response which is represented by a decrement of resistance followed by an increment of resistance in two distinct waves, the second being much weaker than the first. The first increment is, in fact, followed by a period of relative quiescence, during which the resistance remains almost constant for 1—2 hours, after which the vitality decreases rapidly, as is shown by the great decrement of the resistance. Fig. 6 is a typical series of readings in the form of a graph. During the final decrement of resistance the plant can still perceive a change to the horizontal, as is proved by the sudden drop in the graph at $-90°$. Sometimes if the root was about 2 cm. long it proved very vigorous and the resistance increased for a considerable time (fig. 7) before the loss of vitality began to show.

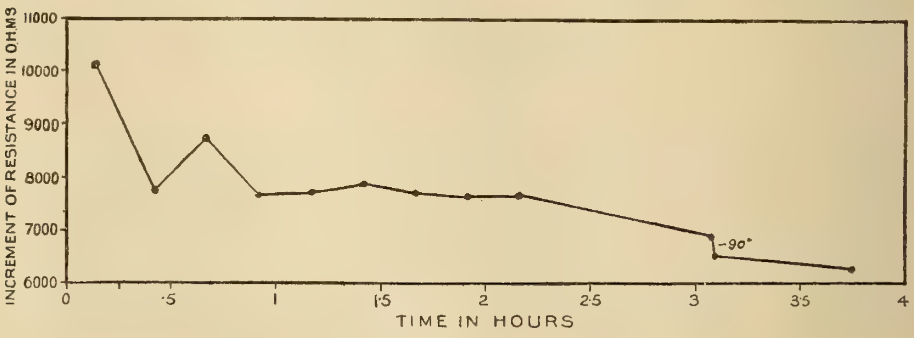

FIG. 6.

The extent of the injury was also tested by growing the beans after they had been used for an experiment. If the readings extended for an hour and a half to two hours, the root afterwards recovered more or less and continued growth with slight abnormalities. If, however, the experiment lasted over 2 hours the side of the root-tip in which the wires had been placed decayed and the growth of the other side produced various twistings. In other cases the whole tip died and numerous lateral roots were produced about 1 cm. behind the apex of the main root, which grew very thick for a distance of 2–3 cm. from the point. In several cases where the wires were left in roots 2–3 cm. long for 48 hours after the experiment, the root grew out of the wax coat and left the wires, being itself apparently unaffected by the injury.

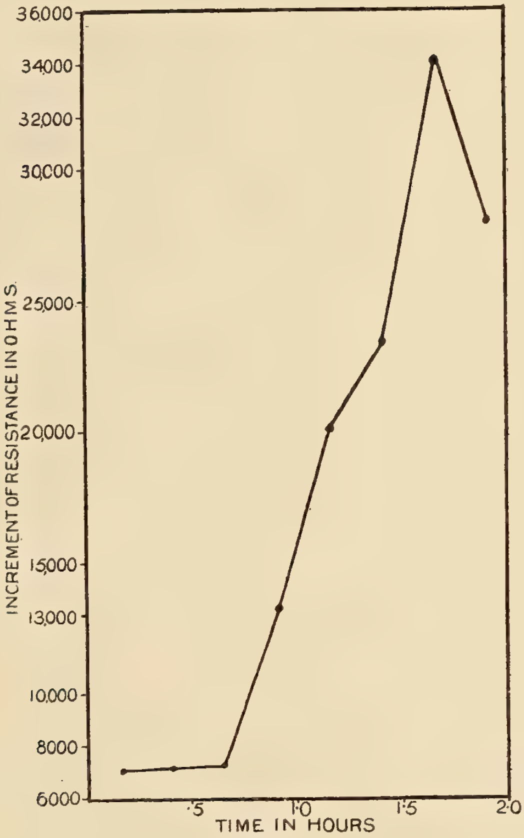

Fig. 7.

The changes in the resistance produced by tilting the root were then studied during the period of quiescence. The plant was mounted and readings taken every 15 min. with the root vertical until two consecutive readings were similar, then the root was placed at various angles with the vertical.

Changes in Resistance and Geotropic Stimulation.

In all the following experiments roots from 2 to 4 cm. in length were used and the beans were germinated upon a vertical board covered with blotting paper and with a cloth arranged over several nails to form a chamber which was kept moist by running water. In this way most of the roots were obtained straight and vertical and the preparatory manipulation was carried out with the root as nearly as possible in the position of rest.

The decrement of resistance furnishes a standard for the measurement of the degree of excitation, instead of the time-ratio used in fig. 1. The resistance was measured with the root vertical at intervals of 15 min. until two similar readings were obtained as explained above, then after another interval of 15 min. the root was tilted at an angle of 20° and a reading taken as quickly as possible. The time taken to get the silence point varied from 15 to 20 sec. As the ratio of the relaxation time to the time of excitation in *Vicia Faba* is 5 : 1,* the root was returned to the vertical position immediately after the reading had been obtained and an interval of 10–15 min. allowed to elapse between each reading. Other angles were taken in succession. The advantage of the present method over previous methods lies in the fact that the behaviour of the same bean root towards different stimuli can be studied; this eliminates to a certain extent that individual variation (*cf.* Tröndle (8)) which complicates the interpretation of most results in geotropism.

The resistance is calculated thus:—$P : R :: AC : CB, \qquad \therefore P = \frac{AC}{AB} \times R.$

Experiment 1 : Fixed resistance or R = 6400 ohms.

Angle of root to vertical ...	0°	−20°	−30°	−45°	−60°	−90°
Total resistance in ohms ...	9600	8660	8145	7667	9600	8839
Decrement of resistance ...	—	940	1455	1933	0	761

It will be seen that the first part of the curve, fig. 8, closely approximates

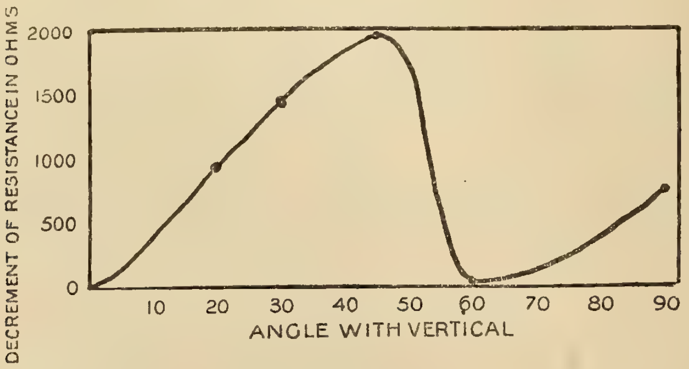

Fig. 8.

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* Fitting (3), Teil II, p. 340.

that in fig. 1. The remainder of the curve shows the injury effects, which are, however, more than counterbalanced by the excitation effects at −90°.

Experiment 2: R = 6000 ohms.

Angle to vertical	0°	0°	−10°	−20°	−30°	−40°	−70°	−90°
Total resistance	7783	7636	7333	6958	6850	6738	6474	6875
Decrement of resistance ...	—	—	303	678	786	898	1162	761

Here the curve is flatter, fig. 9, but the sigmoid character is quite obvious.

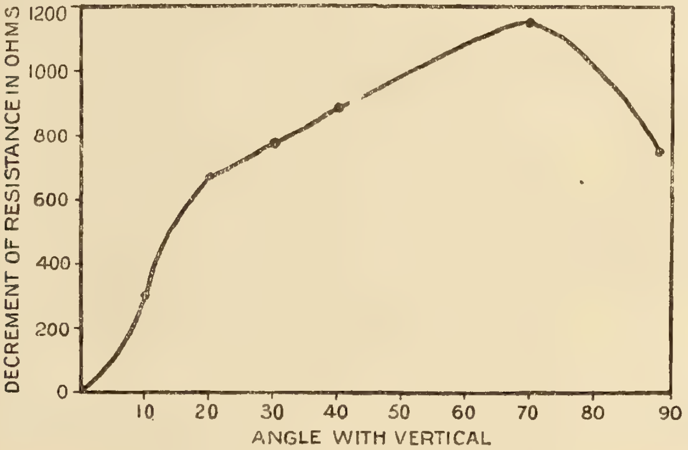

Fig. 9.

Experiment 3: R = 10,000 ohms.

Angle to vertical	0°	0°	−5°	−10°	−20°	−30°	−40°
Total resistance	10,325	10,325	10,040	9417	8869	8691	8552
Decrement of resistance ...	—	—	285	908	1456	1634	1773
Angle to vertical	−45°	−50°	−60°	−70°	−80°	−90°	+90°
Total resistance	8484	8348	7825	7211	7111	6751	7007
Decrement of resistance ...	1841	1977	2500	3114	3214	3574	3320

Although not regularly sigmoid the curve in this case (fig. 10), even after 5 hours, shows a perception of the difference between −90° and +90°. The curve up to −40° is quite regular and as 2¼ hours had then elapsed from the commencement of the experiment the irregularities are quite comprehensible.

The upper half of the curve was investigated in the next two experiments.

Experiment 4: R = 4000 ohms.

Angle with vertical ...	0°	0°	−45°	−50°	−60°	−70°	−80°	−90°
Total resistance	3767	3767	3561	3476	3462	3421	3407	3407
Decrement of resistance ...	—	—	206	291	305	346	367	367

This curve (fig. 11), although the actual reduction is less than in most experiments, confirms the sigmoid character of the curve. Variation is to be expected with the physiological variation of the individuals (*cf.* Tröndle (8)), and the difference between the *percentage* reductions is much less variable, *i.e.* 5–15 per cent.

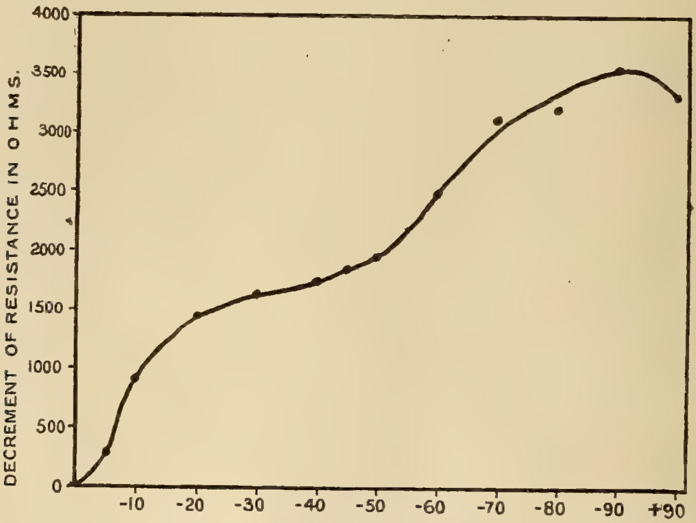

Fig. 10.

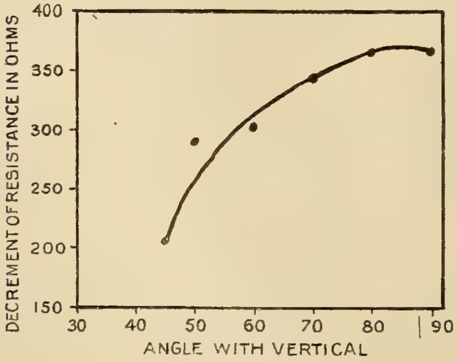

Fig. 11.

Experiment 5 : R = 10,000 ohms.

Angle with vertical ...	0°	0°	−45°	−50°	−60°	−70°	−80°	−90°
Total resistance	12,321	12,222	10,493	10,040	9802	9531	9267	9342
Decrement of resistance	—	—	1,729	2,182	2420	2691	2955	2880

The curve of these results (fig. 12) closely approximates the upper part of the curve in fig. 10 and displays the sigmoid character again.*

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* Loeb and Osterhout (6) agree with Waller (9) in regarding the Weber-Fechner Law as governing many phenomena of stimulation, and Osterhout has suggested a dynamical explanation of the law,

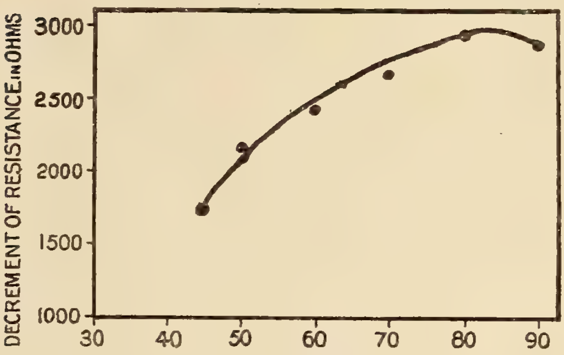

FIG. 12.

Differential Permeability of Upper and Under Sides.

Preliminary experiments indicated that the permeability of both upper and under sides of the root-tip increased, and this was further investigated in the following experiments.

Experiment 6: R = 7000 ohms.

Angle with vertical ...	0°	0°	−10°	+10°	−20°	+20°
Total resistance	8696	8730	8217	8418	7958	8250
Decrement of resistance ...	—	—	513	312	772	480

Experiment 7: R = 6000 ohms.

Angle with vertical ...	0°	0°	−45°	+45°	−70°	+70°	−90°	+90°
Total resistance	6073	6073	5194	5765	4851	5606	4753	5584
Decrement of resistance ...	—	—	879	308	1222	467	1320	489

The results of Experiments 6 and 7 are graphed in fig. 13. The relative flatness of the upper part of the curve is again demonstrated, especially in Experiment 7, where the difference between −90° and +90° is distinctly greater than that between −70° and +70°. Therefore, although the actual permeability is greater at 90°, and the turgor as a consequence less, than at smaller angles with the vertical, the difference between the turgor of the upper and under sides is greater and it is this differential permeability that produces the curvature. This explains the stronger curvatures obtained with the root horizontal.

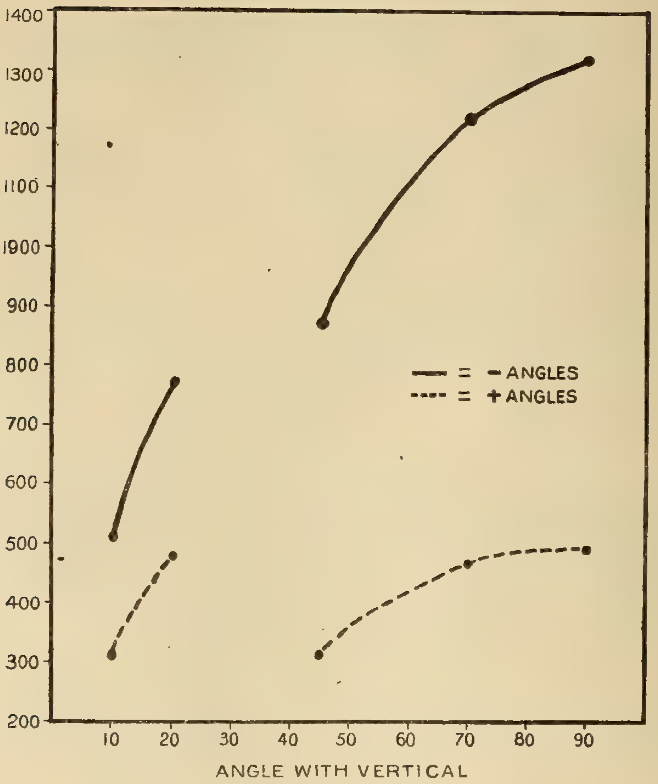

Fig. 13.

Diffusion Effects.

One of the results of increased permeability is more rapid diffusion of salts and a consequent dilution of the cell sap. Such dilution would increase the resistance and was investigated in the following experiments.

Experiment 8 : R = 7000 ohms.

Angle with vertical	0°	0°	−90°	−90°	−90°	−90°	−90°
Total resistance	8283	8318	6958	7345	7645	7798	7798
Decrement of resistance	—	—	1360	1073	673	520	520
Interval in mins.	—	15	2	8	5	5	15

Angle with vertical	−90°	−90°	−90°	−90°	−90°	−90°
Total resistance	7738	7171	7026	6673	6645	6539
Decrement of resistance	580	1147	1292	1645	1673	1779
Interval in mins.	10	15	15	15	45	15

Experiment 9 : R = 7000 ohms.

Angle with vertical	0°	0°	+90°	+90°	+90°	+90°	+90°	+90°
Total resistance	9365	9356	8660	8802	9018	9090	9128	8521
Decrement of resistance	—	—	696	554	338	266	228	735
Interval in mins.	—	15	2	8	6	5	15	10

Angle with vertical	+90°	+90°	+90°	+90°	+90°	+90°	+90°
Total resistance	7863	7767	7705	7584	7584	7614	7404
Decrement of resistance	1493	1589	1651	1772	1772	1742	1952
Interval in mins.	15	15	15	15	15	15	15

It will be seen from the graphs (fig. 14) that the turning of the root horizontal results in a considerable increase of permeability in both cases, the increase being much less when the root was turned so that the side which was being investigated was uppermost. Different beans were used, however, one for Experiment 8 and another for Experiment 9. The root was left in the horizontal position for the remainder of the experiment and the dilution due to diffusion is represented by the downward curves, these giving an increment of resistance from that of the root when it was first turned horizontal. The decrement of resistance due to loss of vitality starts 15–30 min. earlier than was usual in the other experiments, where no time was given for diffusion to take place as the plant was returned to the vertical immediately after each reading.

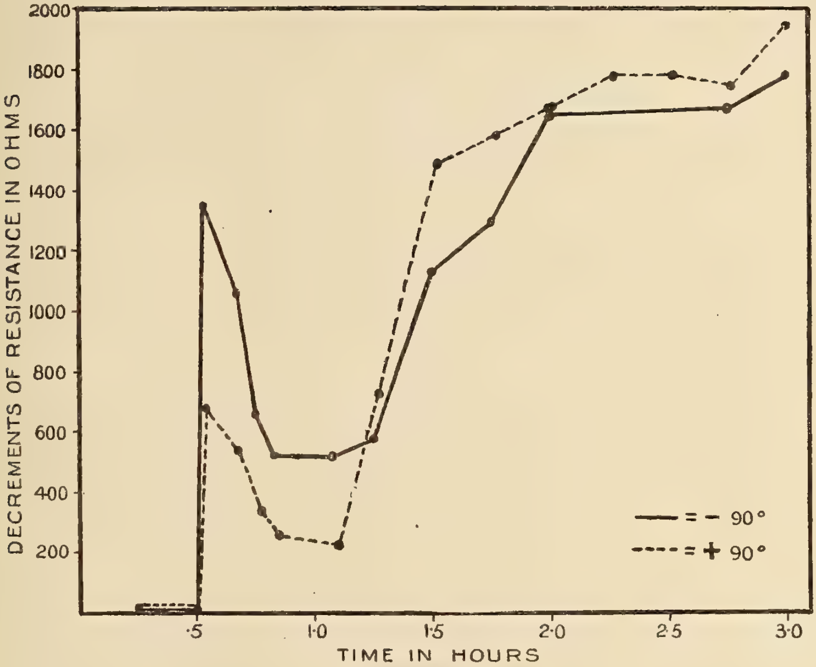

Fig. 14.

Conclusions.

From the time of M. C. Bose (2) to the time of J. C. Bose (1) and Fitting (3) very little advance had been made in the explanation of geotropism. J. C. Bose added to the experimental proof that geotropism is due to protoplasmic action, and interpreted his results as proving a differential change in the turgor on the concave and convex sides of the curve in stems and roots.

He also carried out experiments which proved that the responsive peculiarities of the root are shared by the shoot.

The present investigation proves that the mechanism of geotropic response by the root depends upon permeability changes. These changes in permeability explain the general curvature, the stronger curvature at 90° to the vertical, and the retardation of the elongation of the axis during curvature (Sachs, 7). The perception of gravity by plants is thus brought into line with the response in animals to the same stimulus. It is suggested that it is a general property of protoplasm to react to stimuli according to the sigmoid relation, and that the perception of gravity by protoplasm in general attains an accuracy of between 3 and 4 per cent. of the total stimulus [*vide* fig. 2 and Weber (10) on the perception of gravity by man with an accuracy of 3·3 per cent.].

SUMMARY.

1. That the perception of gravity by the root is a protoplasmic phenomenon is proved by the record of the changes in the electrical resistance of the second millimetre of one side of the root-tip of *Vicia Faba* at various angles to the vertical. These changes show the same sigmoid curve as is shown by animal tissue in response to stimuli.

2. The permeability of the cortical cells of both the upper and the under sides of the root-tip increases when the root is placed at an angle with the vertical, but that of the underside does so to a greater extent. The consequent relatively greater turgidity of the cells of the upper side explains the curvature. The increase in permeability, *i.e.*, decrease in turgor and electrical resistance, of the upper and under sides increases with the angle to the vertical, but the permeability of the upper side increases at a slower rate than that of the under side; this explains the stronger curvature when the plant is horizontal (*vide* fig. 13).

3. The increased permeability, giving decreased turgor, on both sides of the root explains the retardation of the elongation of the axis shown by Sachs (7) to take place during curvature.

In conclusion, I would record my indebtedness to my colleagues of the Chemical, Physiological, Physical and Psychological Departments at Bedford College, especially to Dr. J. S. Edkins and Dr. J. F. Spencer, for valuable assistance and advice.*

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* It is obviously desirable to confirm the results obtained with the telephone method by the galvanometer, for two reasons : (1) the induction currents used in the telephone method produce alterations of resistance ; (2) the subjective determination of the minimum-sound point is sometimes dubious and cannot be demonstrated to an audience. Experiments with the galvanometer are in progress but are not yet completed.

PROCEEDINGS
OF
THE ROYAL SOCIETY.

Series B. Vol. 90. No. B 631.

BIOLOGICAL SCIENCES.

CONTENTS.

Page

Researches on Growth and Movement in Plants by Means of the High Magnification Crescograph. By Sir JAGADIS CHUNDER BOSE, assisted by GURUPRASANNA DAS, L.M.S. 364

Investigations dealing with the State of Aggregation. Part IV.—The Flocculation of Colloids by Salts containing Univalent Organic Ions. By S. B. SCHRYVER, D.Sc., and NITA E. SPEER 400

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HARRISON & SONS, 44—47, ST. MARTIN'S LANE, LONDON, W.C. 2.
Price Three Shillings and Sixpence.

No. B 631.

February 17, 1919.

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[*April* 19, 1918.—Experiments with the galvanometer have been carried out in Dr. Waller's laboratory, but the results with that apparatus were vitiated by a temperature effect due to the moist, non-polarisable electrodes used. It is important, however, that the subjective Kohlrausch method should be supplemented by an objective method, and the writer hopes to develop a suitable apparatus for the necessary confirmation of the results given above.]

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Researches on Growth and Movement in Plants by Means of the High Magnification Crescograph.

By Sir JAGADIS CHUNDER BOSE, assisted by GURUPRASANNA DAS, L.M.S.

(Communicated by Prof. S. H. Vines, F.R.S. Received October 19, 1917.)

The auxanometers usually employed for recording longitudinal growth allow a magnification of only 10 or 20 times. The growth of a plant is, however, so slow that it requires several hours to detect the normal rate of growth and its variation under any changed condition. It is, moreover, impossible to maintain the external conditions absolutely constant throughout the experiment; even if this were possible, there would be some autonomous variation of the rate of growth during such lengthy periods. Hence, there must always be some uncertainty in the results obtained by a method which requires long time for observation. The elements of uncertainty can only be eliminated by reducing the period of the experiment to a few minutes, but that would necessitate devising a method of very high magnification and automatic record of the magnified rate of growth.

I attempted to solve this problem by the employment of the optic lever, where an axis carrying a mirror underwent rotation proportional to the growth-elongation. The reflected spot of light magnified the movement of growth from 1000 to 10,000 times. The vertical movement of the spot of light was converted into a horizontal movement by means of a mirror suitably inclined. The excursion of the spot of light was followed by means of a pen on a drum revolving at a known rate; or the record was obtained automatically by photography. Hence a curve was obtained whose ordinate gave growth-movement, and abcissa time.*

Records thus obtained opened out a very extensive field of investigation on growth and its variations under the manifold influences of environment. The photographic method was automatic, but necessitated the discomfort and inconvenience of a dark room; the results, moreover, could not be followed visually. The other method of obtaining the curve of growth by following the excursion of the spot of light with a pen was far more convenient, but the results in this case are likely to be affected by personal error. In order to obviate all these difficulties I devised a direct method, in which the plant by its own autographs exhibits the absolute rate of growth and the induced variations in an extremely short period of time. I propose, in this paper, to give an account of some of the researches which I have been carrying out by this new method for the last six years; the experiments have been repeated year after year on a variety of plants and with consistent results.

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* Bose, 'Plant Response,' 1906, p. 421.

A. THE HIGH MAGNIFICATION CRESCOGRAPH.

1. Method of high magnification.
2. Automatic record of the rate of growth.
3. Experimental adjustments.
4. Determination of latent period and time-relations of response to stimulus.

B. EFFECT OF INDIRECT STIMULATION.

5. Mechanical and electrical response.
6. Effect on growth.

C. TROPIC AND NASTIC MOVEMENTS.

7. General considerations.
8. Tropic curvature with longitudinal transmission of stimulus.
9. Geotropism.
10. Tropic curvature with transverse transmission of stimulus.
11. Mechanotropism; twining of tendrils.
12. Galvanotropism and electrotropism.
13. Thermonasty.
14. Positive phototropism.
15. Dia-phototropism and negative phototropism.
16. Radio-thermotropism.
17. Photonasty.

D. RELATION BETWEEN RESPONSE TO STIMULATION OF NON-GROWING AND OF GROWING ORGANS.

18. Similarity of response of growing and non-growing organs.

A. THE HIGH MAGNIFICATION CRESCOGRAPH.

I secured high magnification by means of a compound system of two or more levers. The plant is attached to the short arm of a lever, the long arm of which is attached to the short arm of the second lever. If the magnification by the first lever be m, and that by the second n, then the total magnification would be mn.

1. *Method of High Magnification.*

The practical difficulties met with in carrying out this idea are very numerous. It will be understood that just as the imperceptible movement is highly magnified by the compound system of levers, the various errors and difficulties are likely to be magnified in the same proportion. The principal difficulties met with were due: (1) to the weight of the compound lever, which exerted a great tension on the growing plant; (2) to the yielding of flexible connections by which the plant was attached to the first lever, and the first lever to the second; and (3) to the friction at the fulcrums.

Weight of the Lever.—As the first lever is to exert a pull on the second, it has to be made rigid. The second lever serves as an index, and can therefore be made of fine glass fibre. To secure rigidity of the first lever large cross-section and consequent weight is required, which exerts considerable tension on the plant. Excessive tension greatly modifies growth; even the weight of the index used in self-recording auxanometers is found to retard the normal rate of growth. The weight of the levers introduces an additional difficulty in the increased friction at the fulcrums on account of which there is an obstruction of the free movement of the recording arm of the lever. The conditions essential for overcoming the various difficulties are therefore: (1) construction of a very light lever possessing sufficient rigidity, and (2) arranging the levers in such a way that the tension on the plant may be reduced to any extent, or even eliminated.

I found in " navaldum," an alloy of aluminium, a light material possessing sufficient rigidity. The lever is constructed out of a thin narrow sheet 25 cm. in length. The first lever has, as explained before, to be fairly rigid in order to exert a pull on the second without undergoing any bending; this rigidity is secured by giving the thin narrow plate of the lever a **T**-shape. The first lever balances, to a certain extent, the second. Finer adjustments are made by means of an adjustable counterpoise B, at the end of the levers. By this means the tension on the plant can be greatly reduced, or a constant tension may be exerted by means of weight T (fig. 1). In my later type of apparatus the plant-connection is made to the right instead of the left side of the first fulcrum. This gives certain practical advantages. The second lever is then made practically to balance the first, only a very slight weight being necessary for exact counterpoise. The reduction of total weight thus secured reduces materially the friction at the fulcrum, with great enhancement of efficiency of the apparatus.

The recording lever has a normal excursion through 8 cm. on the recording surface, which is a very thin sheet of glass 8 × 8 cm., coated with a thin layer of smoke. As the recording lever is about 40 cm. in length, the curvature in the record is slight, and practically negligible in the middle portion through a length of 4 cm. The dimensions given allow a magnification of 10,000. A far more compact apparatus is made with 15 cm. levers. This gives a magnification of 1000.

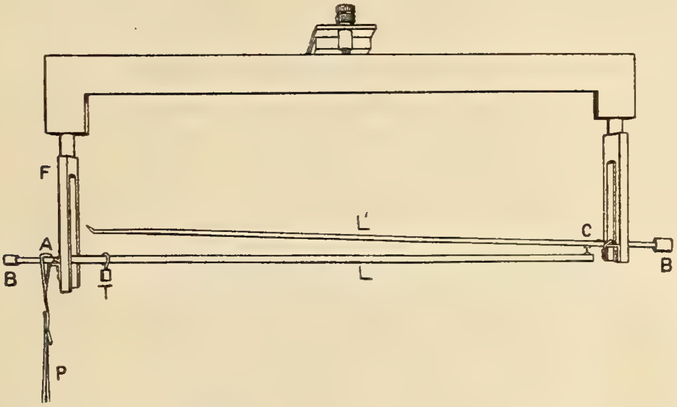

FIG. 1.—Compound lever. P, plant attached to short arm of lever, L ; T, weight exerting tension ; C, connecting link ; L′, second lever with bent tip for tracing record. BB, balancing counterpoise ; Fork, F, carrying on its sides two conical agate cups, on which lever rests by two pin-points.

Connecting Links.—Another puzzling difficulty lay in the fact that the magnification actually obtained was sometimes very different from the calculated value. This unreliability I was able to trace to the defects inherent in the thread-connections at first employed to attach the plant to the first lever and the first lever to the second. These flexible connections were found to undergo variable amounts of elastic yield. Hence it became necessary to use nothing but rigid connections. The plant-attachment, A, of triangular shape is made of a piece of navaldum ; its knife-edge rests in a notch on the short arm of the lever, L. There are several notches at various distances from the fulcrum. It will be understood how the magnification can be modified by moving A nearer to or further from the fulcrum. The lower end of the attachment is bent in the form of a hook. The end of the leaf of the plant P is doubled on itself and tied. The loop thus formed is then slipped over the hooked end of A.

The link, C, connecting L and L′ (fig. 1) consists of a pin pointed at both ends, which rests on two conical agate cups fixed to the upper and lower surfaces respectively of the levers L and L′. This mode of frictionless linking is rigid and allows at the same time perfectly free movement of the levers.

The Fulcrum.—The most serious difficulty was in connection with frictionless support of the axes of the two levers. The horizontal axis was at first supported on jewel bearings, with fine screw adjustment for securing lateral support. Any slight variation from absolute adjustment made the bearing either too loose or too tight, preventing free play of the lever. When perfect adjustment had been secured, the movement of the levers became jerky after a few days. This I afterwards discovered was due to the deposit of invisible particles of dust on the bearings. There were frequent interruptions of work on account of the break of the fine points in the pivot, which have to bear considerable strain in a lateral direction. These difficulties forced me to work out a very perfect and at the same time a much simpler device. The lever now rests on two fine vertical pin-points on conical agate cups carried by the fork F. The axis of the lever passes through the points of support. The friction of support is thus reduced to a minimum and the lever is kept in place under the constant pressure of its own weight. The excursion of the recording end of the lever, which represents magnified movement of growth, was now found to be without jerk and quite uniform.

It is possible to construct a still more sensitive instrument by means of a compound system consisting of *three* levers. There is, however, a limit to the number of levers that may be employed with advantage: for the slight overweight of the last lever becomes multiplied and exerts great tension on the plant, thus interfering with its normal growth.

2. *Automatic Record of the Rate of Growth.*

Another great difficulty in obtaining an accurate record of the curve of growth arises from the friction of contact of the bent tip of the writing lever against the recording surface. This I was able to overcome by an oscillating device in which the contact, instead of being continuous, is made intermittent. The smoked glass plate, G, is made to oscillate to and fro at regular intervals of time, say one second. The bent tip of the recording lever comes periodically in contact with the glass plate during its extreme forward oscillation. The record would thus consist of a series of dots, the distance between successive dots representing magnified growth during a second.

The drawback in connection with the obtaining of a record on the oscillating plate lies in the fact that if the plate approaches the recording point with anything like suddenness, then the stroke on the flexible lever causes an after-oscillation; the multiple dots thus produced spoil the record. In order to overcome this, a special contrivance is necessary, by which the speed of approach of the plate should be gradually reduced to zero at contact with the recording point. The rate of recession should, on the other hand, continuously increase from zero to maximum. The recording point will in this manner be gently pressed against the glass plate, marking the dot, and then gradually set free. It is only by strict observance of these conditions that the disturbing effect of after-vibration of the lever can be obviated.

This particular contrivance consists of an eccentric rod actuated by a rotating wheel. A cylindrical rod is supported eccentrically, so that semi-rotation of the eccentric causing a pull on the crank K (fig. 2) pushes the plate-carrier gradually forward. On the return movement of the eccentric, a light antagonistic spring makes the plate recede. The rate of movement of the crank itself is further regulated by a revolving wheel which is released periodically by clockwork at intervals of 1, 2, 5, 10, or 15 seconds respectively, according to the requirements of the experiment. The complete apparatus is shown in fig. 3.

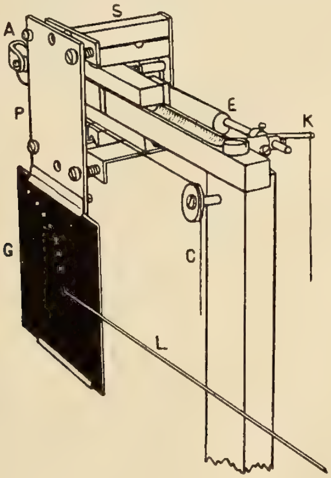

Fig. 2.—Eccentric for oscillation of plate; K, crank; S, slide; P, holder for glass plate G; A, adjusting screws; L, recording lever. Clock releases string, C, for lateral movement of the plate.

I used at first a pair of parallel eccentrics, but in the newest type of apparatus with improved to-and-fro sliding arrangement, one eccentric is found to be quite sufficient. A very important condition for success is the securing of perfect smoothness of movement during the oscillation of the plate. A horizontal slide, moving on ball-bearings, carries the vertical plate-holder. The slide is so perfect in action that a puff of air is by itself sufficient to move the free plate-carrier either backward or forward. The plate may thus be maintained in its to-and-fro oscillation with very little expenditure of force, and the power drawn from the wound-up clock is therefore very small. I have recently been experimenting with an electric oscillating device, which simplifies the matter still further. Electric current flows intermittently through a coil of wire which sucks in a rod of soft iron attached to the plate-carrier. The force required for bringing about the oscillatory movement thus acts directly, without any intervention of the eccentric.

The amplitude of oscillation of the plate is about 3 mm. It is important that the vertical recording plate should be so adjusted that its distance from the recording tip should remain the same during the excursion of the index or during the lateral displacement of the plate moved by clockwork. Failure to secure this makes the dot-marks unequally distinct; in the worst cases some of the dots may even be missing. The difficulty is obviated by accurate adjustment of the plate in a vertical plane by means of regulating screws.

With the particular connection and arrangement of levers the recording tip comes down with growth-elongation. It is perhaps more natural to associate upward growth with an up-curve. The record can, however, be easily reversed by turning over the plate and inspecting the dots from the unsmoked side of the plate.

3. *Experimental Adjustments.*

Adjustment of Tension.—I have explained how the tension exerted on the plant could be rendered negligible. In other cases it may be desirable to apply a definite tension or to increase or decrease it in a quantitative manner. Normally, the levers are so adjusted by suitable counterpoise, as to bring the recording tip to the zero position, which is half-way up the plate. A weight of, say, 1 grm. is now placed on the attachment A (fig. 1) and a weight, T (found by experiment), placed on a fixed notch in L, so as to bring the recording tip once more to zero position. It is clear that when the plant is attached to A, T exerts on it a tension of 1 grm. Various weights are in a similar manner employed to exert tension on the plant from 1 to 10 grm. The tension generally used is about 3 grm.

Determination of the Magnifying Power.—There are two micrometric adjustments, S and S′ (fig. 3), one rough and the other fine, by which the plant may be raised or lowered and the recording tip brought to any part of the glass plate. With the connection of the levers shown in fig. 1, the upward growth of the plant lowers the right arm of the lever L, and the recording tip of the second lever L′ also moves downwards with the growth of the plant. To determine the magnifying power, a fine black dot is made at the point of attachment of lever and plant, and a particular division of the scale of a microscope-micrometer is focussed to the mark. By means of the micrometer screw, S, a fixed rod attached to the first lever is lowered till the mark is moved through, say, 1 mm. The magnified movement of the other end of the lever at C is measured on a scale. By careful adjustment of the distance of attachment A, from the fulcrum, the magnification of the first lever is made about 100 times. The same procedure is followed to obtain the magnifying power of the second lever. Final adjustments are so made that the actual compound magnification obtained is 10,000 times. In other cases the magnifications employed are 5000, 2000, and 1000.

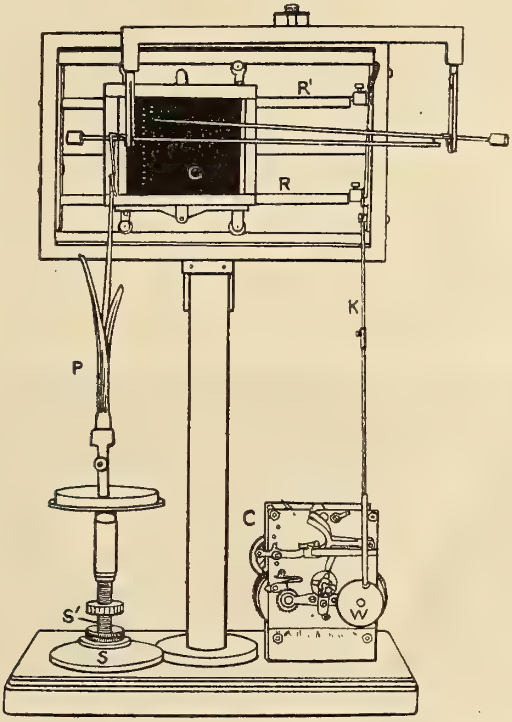

Fig. 3.—Complete apparatus. P, plant; S, S′, micrometer screws for raising or lowering the plant; C, clockwork for periodic oscillation of plate; W, rotating wheel.

Experimental Accessories.—The soil in a flower pot is liable to be disturbed by irrigation and the record thus vitiated by physical disturbance. This is obviated by wrapping a piece of cloth round the root imbedded in a small quantity of soil. The lower end of the plant is held securely by the clamp of the plant-holder. In order to subject the plant to the action of gases and vapours, or to variation of temperature, it is enclosed in a cylindrical chamber constructed of a sheet of mica. The chamber is maintained in a humid condition by means of a sponge soaked in water. To study the direct and after-effects of vapours and gases, the vessel V is filled with the given vapour and the second vessel V′ with air or water-vapour (fig. 4). The plant-chamber is filled with a given gas by the working of bellows and the manipulation of the key K; after a given time the gas is replaced by normal air.

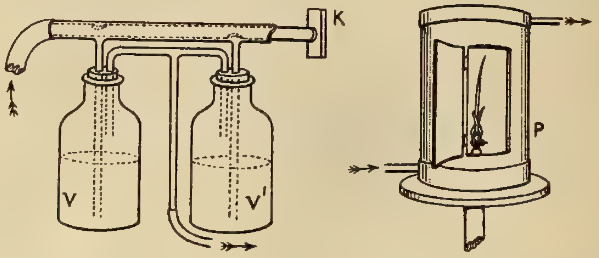

Fig. 4.—P, plant-chamber; V, V′, vessels for introducing into the plant-chamber hot or cold water-vapour, different gases or air, by manipulation of key, K.

For variation of temperature the two vessels are filled, one with hot and the other with cold water. The plant may thus be subjected to a given variation of temperature, by the injection of hot or cold water-vapour into the plant-chamber.

Any quick-growing organ of a plant will be found suitable for experiment. In order to avoid all possible disturbing action of circumnutation, it is preferable to employ either radial organs, such as flower peduncles and the buds of certain flowers, or the limp leaves of various species of grasses and the pistils of flowers. It is also advisable to select specimens in which the growth is uniform. I give below a representative list of various specimens in which, under favourable condition, the rates of growth are of the following order:—

Peduncle of Crocus (*Zephyranthes tubispatha*, Herb.)	0·05 mm. per hour.
Flower bud of *Crinum*	0·07 ,, ,,
Leaf of grass	1·10 ,, ,,
Pistil of *Hibiscus* flower	1·20 ,, ,,
Seedling of wheat	1·60 ,, ,,
Seedling of *Kysoor*	3·00 ,, ,,

A specimen which I found very suitable for experiments on growth is a Cyperaceous plant, *Scirpus Kysoor*, Roxb., locally known as *Kysoor*. The leaves are much stronger than those of wheat and different grasses, and can bear considerable amount of pull without harm. Its rate of growth under favourable condition of season is considerable. Some specimens were found to have grown more than 8 cm. in the course of 24 hours or more than 3 mm. per hour. This was during the rainy season in the month of August, but a month later the rate of growth fell to about 0·8 mm. per hour.

I will now proceed to describe certain typical experiments which will show: (1) the extreme sensibility of the crescograph; (2) its wide applicability to different investigations; and (3) its capability of determining with great precision the time-relations of responsive changes in the rate of growth. In describing these typical cases, I give a detailed account of the experimental methods employed, and thus avoid repetition in describing subsequent experiments.

Expt. 1. *Determination of the Absolute Rate of Growth.*—I will describe the results of a record on a stationary plate obtained with *Kysoor.* The oscillation-frequency of the plate was once in a second, and the magnification employed was 10,000 times. The magnified growth-movement was so rapid that the record consists of a series of short dashes instead of dots (fig. 5, *a*).

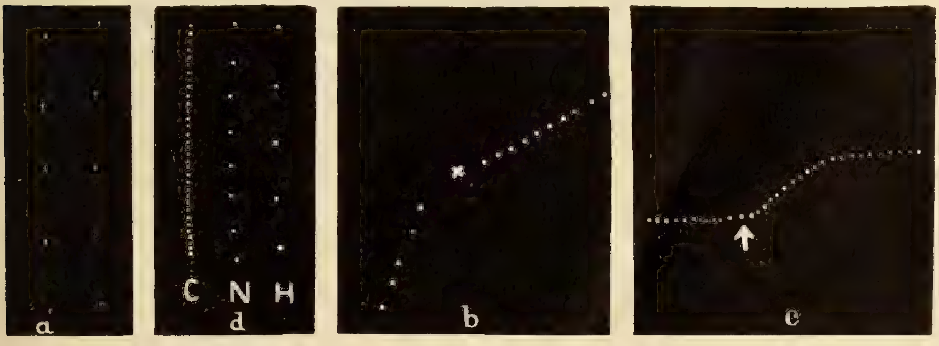

Fig. 5.—Crescographic records. *a*, successive records of growth at intervals of 1 second: × 10,000, with a stationary plate. Effect of temperature: *d*, N, normal rate of growth; C, retarded rate under cold; H, enhanced rate under warmth; *b*, record on moving plate, where diminished slope of curve denotes retarded rate under cold; *c*, horizontal record showing absence of growth in dead branch; physical expansion on application of warmth at arrow followed by horizontal record on attainment of steady temperature. × 2000.

For securing regularity in the rate of growth, it is advisable that the plant should be kept in uniform darkness or in uniformly diffused light. So sensitive is the method of record that it shows a change of growth-rate due to the slight increase of illumination by the opening of an additional window. One-sided light, moreover, gives rise to disturbing phototropic curvature. With the precautions described, the growth-rate in vigorous specimens is found to be very uniform.

After the completion of the first vertical series, the recording plate was moved 1 cm. to the left; the tip of the recorder was brought once more to the top by means of the fine screw adjustment S (fig. 3), and the record taken once more after an interval of 15 minutes. The magnified record for

4 seconds is 38 mm. in the first record. It is precisely the same in the record taken 15 minutes later. The successive growth-elongations at intervals of 1 second are practically the same throughout, being 9·5 mm. This uniformity in the spacings demonstrates not only the regularity of growth under constant conditions, but also the reliability and perfection of the apparatus. It also shows that by keeping the external conditions constant, the normal growth-rate could be maintained uniform for at least 15 minutes. As the magnified growth is nearly 1 cm. per minute, and as it is quite easy to measure 0·5 mm., the crescograph enables us to record a length of 0·00005 mm., that is to say, the sixteenth part of a wave of red light. The absolute rate of growth, moreover, can be determined in a period as short as 0·05 second. These facts will give us some idea of the enormous possibilities of the crescograph for future investigation.

As the period of the experiment is very greatly shortened by the method of high magnification, I shall, in the determination of the absolute rate of growth, adopt a second as the unit of time and μ or micron as the unit of length—the micron being 0·000001 metre, or 0·001 mm.

If m be the magnifying power of the compound lever, and l the average distance between successive dots in millimetres at intervals of t seconds, then

$$\text{rate of growth} = \frac{1}{mt} \times 10^3\mu \text{ per sec.}$$

In the record given $l = 9{\cdot}5$ mm., $m = 10{,}000$, $t = 1$ second.

Hence rate of growth

$$= \frac{9{\cdot}5}{10{,}000} \times 10^3\mu \text{ per sec.} = 0{\cdot}95\ \mu \text{ per sec.}$$

Expt. 2. *Precaution against Physical Disturbances.*—There may be some misgiving about the employment of such high magnification; it may be thought that the accuracy of the record might be vitiated by physical disturbance, such as vibration. In physical experimentation far greater difficulties have been overcome, and the problem of securing freedom from vibration is not at all formidable. The whole apparatus need only be placed on a heavy bracket screwed on the wall to ensure against mechanical disturbance. To what extent this has been realised will be found from the inspection of the first part of the record in fig. 5, *c*, taken on a moving plate. A thin dead twig was substituted for the growing plant, and a perfectly horizontal record not only demonstrated the absence of growth-movement but also of all disturbance. There is also another element of physical change, against which precautions have to be taken in experiments on variation of the rate of growth with rising temperature. In order to determine its character and extent, a record was taken, with the dead twig, of the effect of raising the temperature of the plant-chamber through 10°. The record, with a magnification of 2000, shows that there was an expansion during the rise of the temperature, after which there was a cessation of physical movement, the record becoming once more horizontal. The obvious precaution to be taken in such a case is to wait for several minutes for the attainment of steady temperature. The movement caused by physical change abates in a short time, whereas the change of rate of growth brought about by physiological reaction is persistent.

Having demonstrated the extreme sensitiveness and reliability of the apparatus in quantitative determinations, I proceed to show its wide applicability for various researches relating to the influence of external agencies in modification of growth. For this two different methods are employed. In the first, the records are taken on a stationary plate; the first in the series gives the normal rate; the second is the record taken under the given changed condition. The increase or diminution of the space between successive dots in the two records at once demonstrates the stimulating or depressing nature of the changed condition. In the second method, the record is taken on a plate moving at a uniform rate by clockwork. A curve is thus obtained, the ordinate representing growth-elongation and the abscissa the time. The increment of length divided by the increment of time gives the absolute value of growth at any part of the curve. As long as growth is uniform, so long the slope of the curve remains constant. If a stimulating agency enhances the rate of growth, there is an immediate flexure in the curve. A depressing agent lessens the slope of the curve.

Expt. 3.—I will now give a few typical examples of the employment of the crescograph for the investigation of growth; the first example I shall take is the demonstration of the influence of temperature.

Stationary Method.—Fig. 5, *d*, gives records taken on a stationary plate. The specimen used was *Kysoor*; the crescograph magnification was 2000, and the successive dots at intervals of 5 secs. The middle series, N, was at the temperature of the room. The next, C, was obtained with the temperature lowered by a few degrees. Finally, H was taken when the plant-chamber was warmed. It will be seen how under cooling the spaces between successive dots have become shortened, showing the diminished rate of growth. Warming, on the other hand, caused a lengthening of the spaces between successive dots, thus demonstrating an enhancement of the rate of growth.

Calculating from the data obtained from the figure, we find:

Absolute value of normal rate	0·457 μ per sec.
Diminished rate under cold	0·101 „ „
Enhanced rate under warmth	0·737 „ „

Moving Plate Method.—Another experiment was carried out with a different specimen of *Kysoor*, the record being taken on a moving plate (fig. 5, *b*). The left part of the curve here represents the normal rate of growth. The plant was then subjected to moderate cooling, and the subsequent curve with its diminished slope denotes the depression of growth.

4. *Determination of Latent Period and Time-Relations of Response to Stimulus.*

Expt. 4.—In the determination of time-relations of responsive change in growth under external stimulus, I take the typical case of the effect of electrical shock of one second's duration from a secondary coil. Two electrodes were applied, one above and the other below the growing region of a bud of *Crinum*. The record was taken on a moving plate, magnification employed being 2000, and successive dots made at intervals of 2 seconds. It was a matter of surprise to me to find that the growth of the plant was affected by an intensity of stimulus far below the limit of our own perception. For convenience I shall designate the intensity of electric shock that is barely perceptible to us, as the unit shock. When an intensity of 0·25 unit was applied to the growing organ, it responded by a retardation of the rate of growth. As regards the relative sensibility of plant and animal, I may say that the leaf of *Mimosa pudica*, in a favourable condition, responds to an electric stimulus which is one-tenth the minimum intensity that causes perception in a human being.*

Inspection of fig. 6, *a*, shows that a flexure is induced in the curve in response to stimulus, the flattening of the curve denoting retardation of growth. The latent period in this case was 6 seconds. The normal rate was found restored after a rest of 5 minutes. The intensity of shock was next raised from 0·25 unit to 1 unit. The second record shows that the latent period was reduced to 4 seconds, and a relatively greater retardation of growth was induced by the action of the stronger stimulus. The recovery of the normal rate was effected after the longer period of 10 minutes. I took one more record, the stimulus being 3 units. The latent period was now reduced to 1 second, and the induced retardation was so great as to effect a temporary arrest of growth, after which there was a slow recovery.

Expt. 5.—As a further example of the capability of the crescograph, I will give the record of a single pulse of growth obtained with the peduncle of Crocus (fig. 6, *b*). The magnification employed was 10,000, the successive dots being at intervals of 1 second. It will be seen that the growth-pulse commences with a sudden elongation, the maximum rate being 0·4 μ per second. The pulse exhausts itself in 15 seconds, after which there is a partial recovery in the course of 13 seconds. The period of the complete pulse is, therefore, 28 seconds. The resultant growth in each pulse is, therefore, the difference between elongation and recovery. Had a highly magnifying arrangement not been used, the resulting rate would have appeared continuous. In other specimens, owing probably to greater frequency of pulsation and co-operation of numerous elements in growth, the rate appears to be practically uniform.

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* Bose, 'Irritability of Plants,' 1913, p. 50.

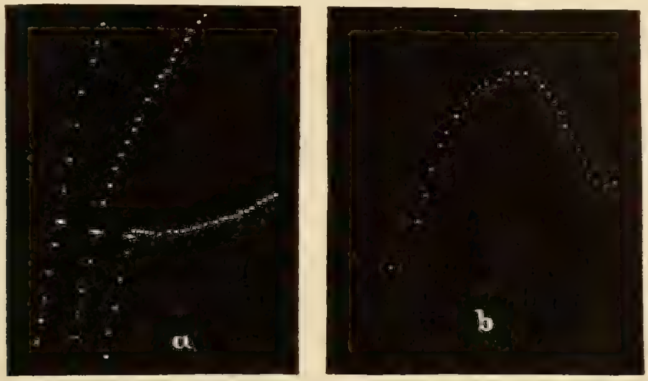

Fig. 6.—*a*, time-relations of response of growing bud of *Crinum* to electric stimulus of increasing intensity applied at the short lines; *b*, record of a single growth-pulse of *Crocus*.

Table I.—Time-relations of Growth-variation under Electric Shock. (*Crinum.*)

Intensity of stimulus.	Latent period.	Normal rate.	Retarded rate.
0·25 unit	6 seconds	0·62 μ per sec.	0·49 μ per sec.
1 unit	4 seconds	0·62 " "	0·25 " "
3 units	1 second	0·62 " "	Temporary arrest of growth.

It is thus found that growth in plants is affected by an intensity of stimulus which is below human perception; that with increasing stimulus the latent period is diminished and the period of recovery increased; that the induced retardation of growth increases continuously with the stimulus till at a critical value there is a temporary arrest of growth.

B. Effect of Indirect Stimulation.

Having ascertained that the direct application of stimulus gives rise in different organs to contraction, diminution of turgor, fall of motile leaf, electromotive change of galvanometric negativity, and retardation of the rate of growth, I proceeded to inquire whether indirect stimulus (that is to say application of stimulus at some distance from the responding organ) gives rise to an effect different from that of direct application.

5. *Mechanical and Electrical Response.*

In experimenting with various sensitive plants like *Mimosa*, *Averrhoa*, and with ordinary plants like *Artocarpus*, I found that indirect stimulation gives rise to a positive or erectile response of the responding leaf or leaflet (indicative of an increase of turgor), often followed by the normal negative response; the interval between the two varies in different cases from a fraction of a second to 30 seconds or more. A probable explanation of the dual impulse is that stimulation causes a local contraction, with expulsion of water from the cells. The positive hydraulic impulse travels quickly. The interval of time that elapses between the application of stimulus and the erectile response of the responding leaf depends on the distance of the point of application and the character of the transmitting tissue; it varies in different cases from 0·6 second to about 40 seconds. The positive is followed by a slower wave of protoplasmic excitation, which causes the excitatory fall. The velocity of this excitatory impulse is about 30 mm. per second in the petiole of *Mimosa*, and about 3 mm. per second in *Biophytum*. The positive followed by the negative thus gives rise to a diphasic response. The excitatory impulse is much enfeebled during transit; the negative impulse may fail to reach the responding organ if the stimulus be feeble, or if the

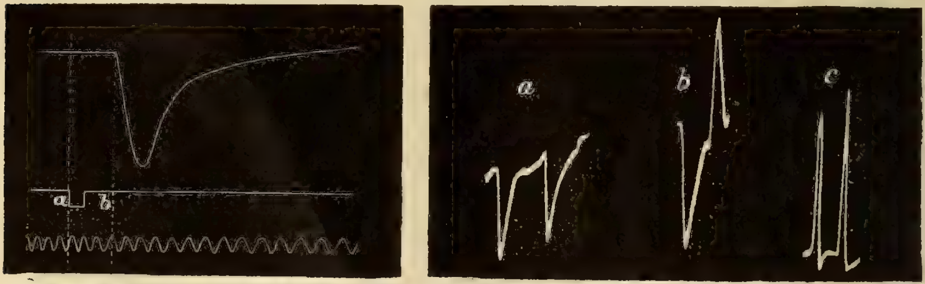

Fig. 7.—Mechanical and electrical response to indirect stimulation. Left, positive response of *Mimosa* : *a*, moment of application of stimulus ; *b*, time-tracings of five vibrations per second. Right, *a*, positive ; *b*, diphasic ; and *c*, negative, electric response of *Musa.*

intervening distance be long or semi-conducting. Hence moderate stimulus applied at a distance gives rise only to positive response; direct application of strong stimulus gives rise, on the other hand, to the normal negative. By employing the electric method of investigation, I have obtained with ordinary tissues the positive, the diphasic, and the negative electric response, in correspondence with the responses given by a motile organ (fig. 7).* The mechanics of propagation of the positive and the negative impulse are different. It is therefore necessary to distinguish the quick *transmission* of the hydro-positive impulse from the slow *conduction* of the negative impulse due to the propagation of excitatory protoplasmic change.

6. *Effect of Indirect Stimulation on Growth.*

I will now describe certain remarkable phenomena connected with growth, the discovery of which was due to the clue obtained from the characteristic response to indirect stimulation that has just been described. I may say here, in anticipation, that the results of experiments which I have carried out on growth-variation under indirect stimulation are precisely parallel to those obtained with non-growing organs. The effect induced by feeble stimulus applied at a distance from the growing region is a positive variation or acceleration of growth, which becomes negative, *i.e.*, retardation of growth, when the stimulus is applied at the responding region of growth; under intermediate conditions, the growth-variation is diphasic, a positive acceleration followed by a negative retardation.

Expt. 6.—Out of these three cases I shall give a detailed account of an experiment relating to the positive variation of growth under moderate indirect stimulation, since this particular case may have some important theoretical significance. I took for experiment a growing bud of *Crinum* and determined the region of its growth activity; lower down a region was found where the growth had already passed its maximum, and may therefore be regarded as an indifferent region. I applied two electrodes on this indifferent region, about 1 cm. below the region of growth. On applying a moderate electric stimulus of short duration, the response was an acceleration of growth which persisted for nearly a minute, after which there was a resumption of the normal rate of growth. In this particular case the interval of time between the application of stimulus and the responsive acceleration of growth was 12 seconds. The interval varied in different cases from 1 second to about 20 seconds, depending on the intervening distance between the point of application of stimulus and the responding region of growth. I also give a record (fig. 8), which shows in an identical specimen (1) an acceleration of growth under indirect, and (2) a retardation of growth under direct stimulation.

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* *Cf.* 'Plant Response,' p. 512; 'Comparative Electrophysiology,' p. 62; 'Irritability of Plants,' p. 176.

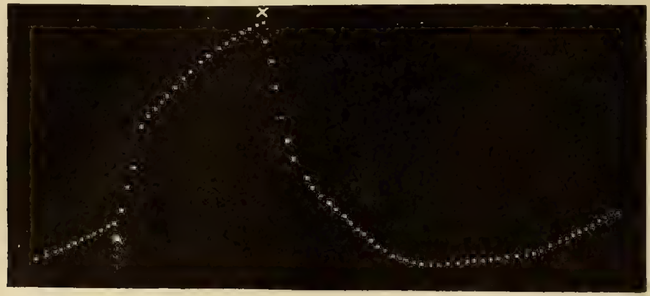

Fig. 8.—Effect of indirect and direct stimulation on growth. (↑) shows application of indirect stimulus with consequent acceleration of growth: application of direct stimulus at (×) induces contraction and subsequent retardation of rate of growth.

Table II.—Accelerating Effect of Indirect Stimulation (*Crinum*).

Specimen.	Rate of growth.	
I	Normal	0·21 μ per sec.
	After indirect stimulation	3·26 ,, ,,
II II	Normal	0·25 ,, ,,
	After indirect stimulation	0·3 ,, ,,

From the above results it will be seen that the effect of stimulus is modified by its point of application.

Hence we arrive at the following laws of direct and indirect stimulation*:—

1. *Direct Stimulation*—

The effect is contraction, diminution of turgor, negative mechanical and electrical response, negative variation (retardation) of the rate of growth.

2. *Indirect Stimulation*—

(*a*) The effect of feeble stimulus is expansion, increase of turgor, positive mechanical and electrical response, positive variation (acceleration) of rate of growth.

(*b*) The effect of prolonged application of stimulus of moderate intensity is a diphasic response, positive mechanical or electrical response followed by the negative; an acceleration, followed by a retardation of growth. If the intervening tissue be highly conducting, the transient positive effect becomes masked by the predominant negative.

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* *Cf.* 'Plant Response,' p. 535; 'Comparative Electro-physiology,' p. 64; 'Irritability of Plants,' p. 196.

It will presently be shown how these fundamental effects of direct and indirect stimulations are instrumental in bringing about various tropic curvatures.

C. TROPIC AND NASTIC MOVEMENTS.

The diverse movements induced by external stimulus in different organs of plants are extremely varied and complicated; the forces in operation are manifold—the influence of changing temperature, the stimulus of contact, of electric current, of gravity, and of light visible and invisible. They act on organs which exhibit all degrees of physiological differentiation, from the radial to the dorsiventral. Stimulus may act on one side or on all sides of the organ. The response may or may not change with the mode of stimulation. In the curving tendril under stimulus of unilateral contact, Fitting finds a pronounced acceleration of growth on the convex side. "Although the exact mode of production of these changes is uncertain, they are undoubtedly the result of the contact stimulus."* Far more complicated are the effects induced by light. Under unilateral stimulation of increasing intensity, a radial organ exhibits a positive, a dia-phototropic and finally a negative response. Strong sunlight brings about para-phototropic or midday sleep movement, by which the apices of leaves or leaflets turn towards or away from the source of illumination. The teleological argument advanced, that in this position the plant is protected from excessive transpiration, does not hold good universally; for under the same reaction the leaflets of *Cassia montana* assume positions by which the plant risks fatal loss of water. In *Averrhoa Carambola* the movement is downwards, whichever side is illuminated with strong light; in *Mimosa* leaflet the movement, under similar circumstances, is precisely in the opposite direction. The photonastic movement, apparently independent of the directive action of light, has come to be regarded as a phenomenon totally unrelated to phototropic reaction, and due to a different kind of irritability and a different mode of response. So very anomalous are these various effects that Pfeffer, after showing the inadequacy of various theories that have been advanced, came to the conclusion that "the precise character of the stimulatory action of light has yet to be determined. . . . When we say that an organ curves towards a source of illumination because of its phototropic irritability, we are simply expressing an ascertained fact in a conveniently abbreviated form, without explaining why such curvature is possible or how it is produced."†

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* Pfeffer, 'Physiology,' vol. 3, p. 58.

† Pfeffer, 'Physiology,' vol. 2, p. 74.

7. *General Considerations.*

The contradictory nature of the various responses is, however, not real; the apparent anomaly lies in the fact that two distinct fundamental reactions to stimulus had hitherto remained undiscovered. The innumerable variations in the resultant response is due to the summation of the effects of two fluctuating factors; it is therefore most important to have the means of tracing the continuous change in the response brought about by the two elements which are sometimes in accord and at other times in conflict. The autograph of the plant itself giving a history of the change in response and its time-relations is therefore decisive in explanation of the various anomalies in plant-movements, as against the various tentative theories that have been put forward. The analysis of the resultant effect thus rendered possible casts new light on responsive characteristics of various organs, such as the localisation of the sensory zone in *Avena,* the illusive difference between tropic and nastic movement, and many other anomalies which are more apparent than real. Limitation of space compels me to confine myself to the consideration of some important and typical reactions which will give clue for the explanation of others.

All the tropic movements under unilateral stimulus find their explanation from the above laws of direct and indirect stimulation that have been established for all modes of excitation. We have henceforth to deal with two classes of effects, due to longitudinal and transverse transmission respectively. In the former case the transmitted stimulus will cause an expansion higher up on the same side to which the stimulus is applied; the result will be an induced convexity, a movement away from stimulus, *i.e.*, a negative curvature. In the case of transverse transmission, the direct effect on the proximal side will be a contraction, and on the opposite side an expansion. When the stimulus is feeble, or when the tissue is a non-conductor of excitation, the first positive will remain as the final effect. But in other cases, conduction of excitation in a transverse direction will bring about a neutralisation or even a reversal into negative. These theoretical deductions I have put to the test of experiment.

8. *Tropic Curvature with Longitudinal Transmission of Stimulus.*

Expt. 7.—I have already explained how thermal radiation is almost as effective in inducing contraction and retardation of growth as the more refrangible rays of the spectrum. The thermal radiation is produced by the heating of a platinum spiral, short of incandescence, by the passage of an electric current. The intensity of radiation is easily varied by adjustment of the current by means of a rheostat. The experimental subject was a flower-bud of growing *Crinum*. It was held by a clamp, a little below the region of growth. Stimulus was applied below the clamp so that the transmitted effect had to pass through the securely held tissue. A feeble stimulus was applied on one side, at the indifferent point about 3 cm. below the region of growth. The indirect stimulus reached the region of growth on the same side, bringing about an acceleration of growth with expansion and convexity, the resulting movement being negative or away from the stimulus. The latent period was 10 seconds, and maximum negative movement was completed in the further course of 10 seconds, after which there was a recovery in the course of 75 seconds. A stronger stimulus gave a larger response; but when the intensity was raised still higher, the hydro-positive was overtaken by the excitatory negative impulse within 15 seconds of the commencement of the positive response; the convex was then succeeded by the concave curvature (fig. 9). I have obtained similar negative and positive curvatures with other plant-organs under various forms of stimuli. Thus the indirect effect of continuous application of light of feeble intensity was to increase negative curvature in a stem of *Dregea volubilis*. Direct application of light on the region of growth gave, on the other hand, a positive curvature.

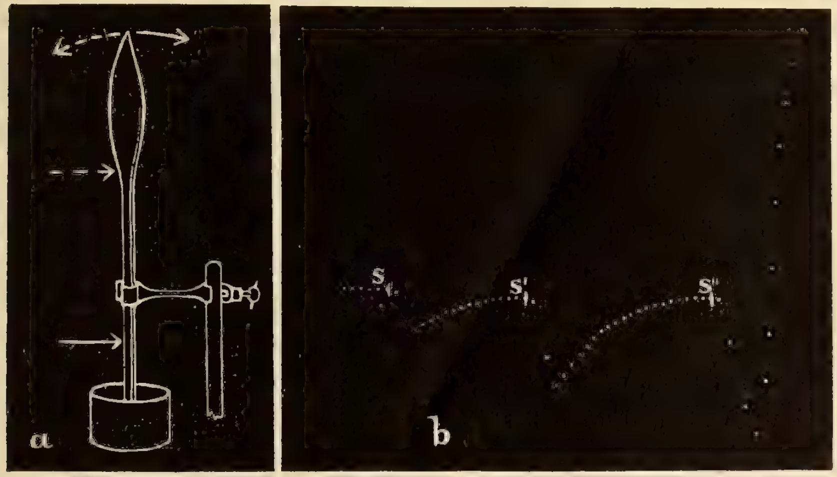

Fig. 9.—Effect of indirect stimulation on growth curvature. *a*, diagrammatic representation: continuous arrow represents indirect stimulation; curved arrow the induced negative curvature; dotted arrow indicates direct application of stimulus; and dotted curve the positive curvature. *b*, indirect stimulation of moderate intensity: S, S′ induced negative tropic effect (movement away from stimulated side); stronger stimulus S″ gave rise to negative followed by positive. (*Crinum.*)

Thus, while the direct effect of unilateral stimulation is to induce a positive curvature, its longitudinal transmission has the indirect effect of inducing a negative curvature.

9. *Geotropism.*

No phenomenon of tropic response is so inexplicable as the opposite effects of stimulus of gravity on the root and the shoot. The experiment that has just been described will, I think, offer an explanation of these diametrically opposite effects. In the root the stimulus is received at the tip and transmitted to the region of growth at some distance; stimulation here is therefore indirect. In contrast with this is the fact that the growing region of the shoot is both sensitive and responsive. Hence the stimulation in this case is direct. As the effects of direct and indirect stimulation on growth are antithetic, the responses of shoot and root to the direct and indirect actions of stimulus must be of opposite signs.

I have carried out other experiments for determining the effects of direct and indirect stimulation on the root itself. Unilateral stimulation of the tip* by thermal radiation induced a negative curvature, while direct stimulation of the growing region brought about a positive curvature.†

These results indicate that there is no necessity for postulating two different irritabilities for the shoot and the root, since tissues in general exhibit positive or negative curvature accordingly as stimulation is direct or indirect.

10. *Tropic Curvature with Transverse Transmission of Stimulus.*

We have next to consider a very large class of phenomena arising out of the direct stimulation of one side, and its transversely transmitted effect on the opposite site. The unilateral stimuli to which the plant is naturally exposed are those of contact, of light, and of thermal radiation. There is besides the stimulation of the electric current. I shall presently show that these tropic curvatures are determined by the definite effects of direct and indirect stimulations. The twining of tendrils by unilateral contact will be taken first, as presenting fewest complications.

11. *Mechanotropism; Twining of Tendrils.*

In experiments on the effect of mechanical friction on growth, I have found that its direct effect is an incipient contraction and retardation of growth; the incipient contraction culminates in an actual contraction under stronger stimulation. Unilateral application of stimulus will bring about a contraction of the proximal side due to the direct effect of stimulation, and an expansion on the distal side due to indirect effect.

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* Darwin's experiments on the curvature brought about by unilateral contact or injury of the root-tip show that other stimuli induce an effect similar to that of gravitation.

† For results of further investigation on the subject *cf.* 'Plant Response,' p. 537.

Expt. 8. *Effect of Diffuse Mechanical Stimulation on the Growth of Tendril.* —I took a growing tendril of *Cucurbita*, and, after obtaining record of its normal rate of growth, subjected it to feeble mechanical stimulation by rubbing its different sides. The immediate effect was a retardation from the normal rate of 0·44 μ per second to 0·20 μ per second. The tendril recovered its normal rate of growth after the feeble stimulation; in fact, the effect, after 15 minutes, was even a slight acceleration above the normal, the growth rate being 0·59 μ per second. The contraction of the directly excited side and the expansion due to indirect stimulation of the distal side will give an explanation of Fitting's observation* that in a unilaterally stimulated tendril there is (1) an acceleration of growth on the convex side, and (2) a contraction on the concave side. Fitting noticed that the tendril became straightened by the active renewal of growth on the excited side.

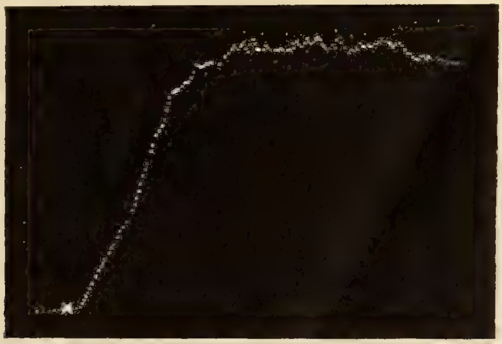

FIG. 10.—Positive curvature of tendril of *Cucurbita* under unilateral stimulus of contact at ×.

I give here a record obtained with a single recording lever (magnification 50 times), showing the response to short unilateral contact in a tendril of *Cucurbita*. Positive curvature was induced in about 15 seconds and attained its climax in 2½ minutes, after which the tendril slowly commenced its recovery, which was completed in 12 minutes (fig. 10). Feeble stimulation is attended by a recovery within a moderate period of time. Under strong stimulation the curvature becomes more persistent.

12. *Galvanotropism and Electrotropism.*

I have demonstrated the retarding effect of the cathode and the accelerating effect of the anode on growth. Unilateral application of anode or cathode will thus bring about appropriate curvatures. The galvanotropic effect may also be demonstrated by the pulvini of different plants such as those of *Erythrina indica.* One electrode is applied on one half of the pulvinus, say the upper half, the second electrode being applied on the stem. On making the pulvinus cathode, a curvature is produced, due to induced contraction and concavity. Reversing the current and making the pulvinus anode causes an opposite curvature due to induced expansion and convexity. An intensity of current above the critical here reverses expansion into contraction.

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* Pfeffer, *ibid.*, vol. 3, p. 57.

Expt. 9.—For obtaining electrotropic response I took a growing bud of *Crinum,* and applied two electrodes on one side of the organ in the growing region. The electrodes were two pins thrust one above the other 1 cm. apart. The effect of mechanical irritation was allowed to pass away. Application of induction-shock of moderate intensity to one flank of the organ gave rise to a positive curvature, the side directly excited becoming concave; there was a recovery after a period of rest.

13. *Thermonasty.*

In the well-known instance of the Crocus flower the effect of rise of temperature is to bring about a relatively quicker rate of growth on one side of the anisotropic organ. This causes the movement of opening; fall of temperature induces the reverse effect of closing.

Expt. 10.—I give below records of the effect of rise and fall of temperature. Some specimens were found extraordinarily sensitive, and the following records give the opposite reactions due to alternate introduction of puffs of cold and warm air. The rise of temperature was so slight that a mercury thermometer did not exhibit any change. Yet the effects of such slight variation of temperature gave rise to marked responses of opposite signs. I

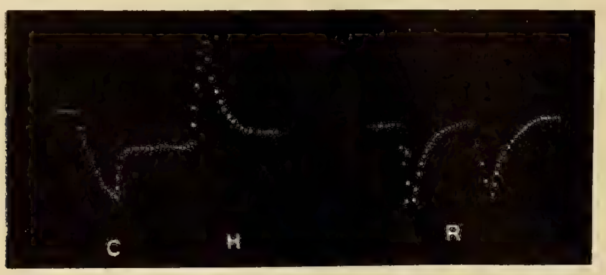

Fig. 11.—Thermonastic and radionastic responses of petal of *Crocus.* C, closing movement due to cooling, and H, opening movement due to warming; R, closing movement due to heat-radiation. Note opposite responses to thermal and radiation action of heat.

here insist once more on the two opposite effects of heat, the *thermal* effect which induces an acceleration and the *radiation* effect which causes a retardation of growth. In order to demonstrate this, I subjected this flower to thermal radiation acting from all sides. The result is a movement which is of opposite sign to the effect of rising temperature (fig. 11).

14. *Positive Phototropism.*

In the study of the responsive curvature induced by unilateral action of light, we have to deal with the joint effects of the contraction of the proximal and the expansion of the opposite side, which would, under normal conditions, give rise to a positive curvature.

By following the electric mode of investigation I have obtained independent corroboration of the characteristic effects of direct and indirect stimulation on the proximal and opposite sides.* The following Table gives the responsive effects induced in pulvini and in growing tissues which act as pulvinoids:—

Table III.—Showing Responsive Effects Common to Pulvini and Growing Organs under Unilateral Stimulation.

Effect of direct stimulation on proximal side.	Effect of indirect stimulation on distal side.
Diminution of turgor Galvanometric negativity Contraction and concavity	Increase of turgor. Galvanometric positivity. Expansion and convexity.
When stimulus is strong or long continued, the true excitatory effect is conducted to the distal side neutralising or reversing the first response.	

When a radial organ is subjected to the unilateral action of light, it exhibits a positive curvature. Fig. 12 gives the positive response of the stem of *Dregea volubilis* to light of short duration from an arc-lamp; the recovery on cessation of light was complete. When the stimulation was stronger, and continued for a longer time, the curvature was greater, and the after-effect was more persistent. The positive curvature does not undergo any modification in the case of thick organs with feeble transverse conductivity, for neutralisation can only take place by the conduction of excitation to the opposite side.

I have said that the normal positive curvature was brought about by the contraction of the proximal and the expansion of the opposite side. I made a surmise that the expansion of the opposite side, due to indirect stimulation, was brought about by an induced increase of turgor.

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* 'Plant Response,' p. 515.

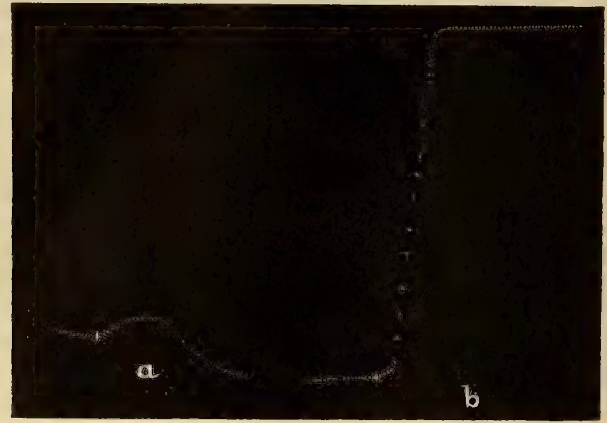

Fig. 12.—*a*, positive phototropic response and recovery from moderate stimulation; *b*, persistent positive curvature under stronger stimulation. (*Dregea.*)

Expt. 11.—It would, undoubtedly, be a matter of great theoretical interest if the induction of enhanced turgor on the distal side (by the action of stimulus at the proximal side) could be demonstrated by some experiment of a convincing character. I have explained elsewhere how enhanced turgor brought about by increased internal hydrostatic pressure caused an erection of the *Mimosa* leaf, a diminution of turgor causing its fall.* I now took a *Mimosa* plant and applied a narrow circular beam of light from a small arc lamp at a point on the stem diametrically opposite to the motile leaf which was to serve as the indicator of induced variation of turgor under the unilateral action of light. That the indirect stimulation caused an enhancement of turgor of the opposite side was soon demonstrated by the erectile movement of the leaf. This positive movement was initiated 20 seconds after the application of stimulus, thus affording a proof, which appears conclusive, of the induction of an increased turgor as the effect of indirect stimulation. When the stimulation is moderate and of short duration, the response is only positive. But when the stimulation is continued, the slow excitatory impulse is conducted to the distal side with immediate fall of the leaf (fig. 13). It was stated that conduction of excitation in a transverse direction would prove very much slower than in the longitudinal direction. In the present case the time taken for conduction across the stem 2 mm. in diameter was 200 seconds, giving a velocity of $\frac{1}{100}$ mm. per second. The velocity along the stem varies in different cases, from one to several millimetres per second. Transverse conductivity is thus about a hundredth of the longitudinal conductivity.

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* 'Plant Response,' p. 46.

Expt. 12.—I next give an account of experiments on the effect of unilateral stimulus of light on the pulvinus of *Mimosa*. The results will be found to be of much theoretical importance, since this single experiment will give an insight into all possible types of phototropic response. I must first remove the general misapprehension that it is the lower half of the pulvinus that is alone excitable. By careful amputation of the lower half I have been able to show that the upper half is also excitable and contracts under electrical stimulation, lifting the leaf. The responsive movement was relatively slower, and the excitability was found to be about one-eightieth that of the lower half.

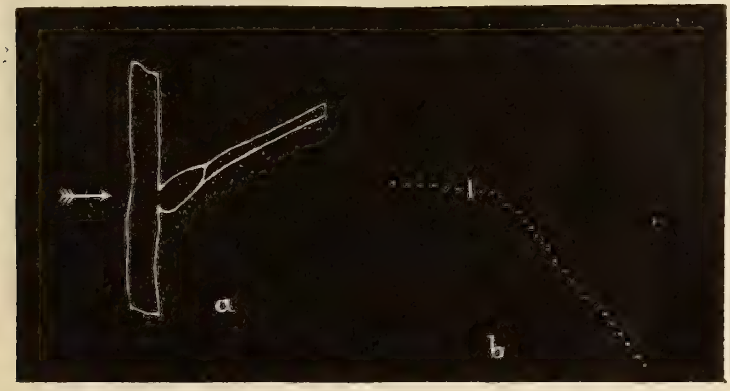


FIG. 13.—Increased turgor due to indirect stimulation, inducing erection of *Mimosa* leaf: *a*, diagram of experiment; *b*, erectile response (shown by down-curve) followed by rapid fall (up-curve) due to transverse conduction of true excitation.

In my present experiment a beam of light from a small arc-lamp was thrown on the upper half of the pulvinus. After a latent period of 5 seconds, a positive curvature was initiated by the contraction of the upper and expansion of the lower side of the organ. A series of *positive* responses may thus be obtained under stimuli of short duration. But under continued action of light the excitatory impulse reaches the lower half of the organ, causing a rapid fall of the leaf, thus constituting a *negative* response (fig. 14). The thickness of the pulvinus was 1·5 mm. The distance to be traversed to reach the lower half would be about 0·75 mm.; the time taken by the excitatory impulse to traverse this distance was found to vary in different cases from 50 to 80 seconds. The transverse velocity is thus about $\frac{1}{80}$ mm. per second.

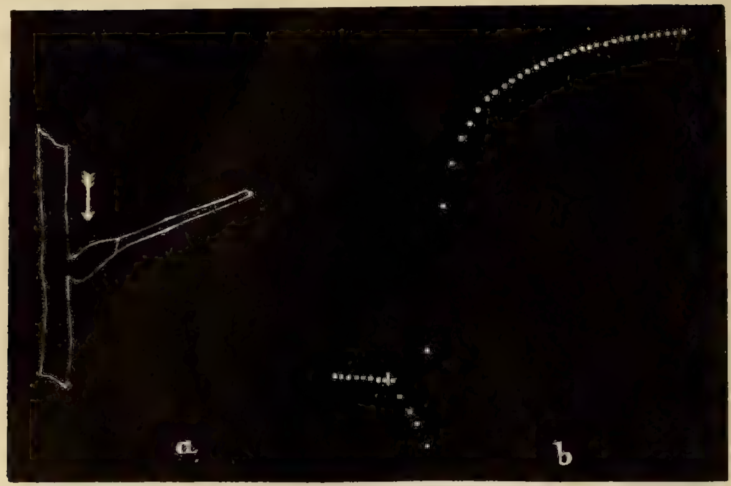


Fig. 14.—Record of effect of continuous application of light on upper half of pulvinus of *Mimosa* leaf. Note erectile response (positive curvature) followed by neutralisation and pronounced reversal into negative due to transverse conduction of excitation. (Up-movement shown by down-curve and *vice versâ*.)

Turning to the main experiment, we find that:—

(1) As a result of the action of light on the upper surface, there was a positive phototropic effect which lasted for 50 seconds.

(2) Owing to the internal conduction of excitation, the positive effect underwent an increasing neutralisation on account of the excitatory contraction of the opposite side. This neutralisation depends on four factors: (*a*) the intensity of the stimulus, (*b*) the conductivity of the organ in a transverse direction, (*c*) the thickness of the intervening tissue, and (*d*) the relative excitability of the opposite as compared with the proximal side. The extent of positive curvature will also depend on the pliability of the organ.

(3) Since the organ exhibits pronounced physiological anisotropy, and the opposite side is far more excitable than the proximal, the internally diffused stimulus brings about a greater contraction of the opposite side. The positive phototropic curvature becomes reversed to a very pronounced negative. The effect of the internally diffused stimulus is thus the same as that of external diffuse stimulation.

(4) When the stimulus is applied on the more excitable side of the organ—in this case the lower half—the result is a predominant contraction of that half; this cannot be neutralised by the excitation conducted to the feebly excitable upper half. As the curvature is towards the stimulus, the phototropic effect will appear to be positive.

Hence, unilateral stimulation of an anisotropic organ will appear to give different results depending on the particular flank that is excited. Stimulation of the less excitable side will give the sequence of positive, neutral, and negative response; stimulation of the more excitable side will give only the positive. The question as to which side is the more excitable can easily be determined by applying to the organ a diffuse electric shock. The more contracted and concave side is the more excitable.

I may now classify some of the principal types of response that will be met in practice. In anisotropic organs, stimulus is supposed to be applied on the less excitable side.

I. Radial organ—

(*a*) Thick stem, transverse conduction negligible: positive phototropic response.

(*b*) Thin stem, transverse conduction possible: sequence of responses—positive, neutral, and negative.

II. Pulvinated organ, motile or non-motile—

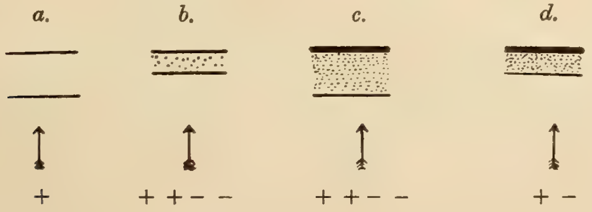
(*a*) Transverse conduction negligible: positive response; pronounced concavity of the excited side, *e.g.*, mid-day sleep or paraphototropism of *Erythrina indica*, *Clitoria ternatea*, and others.

(*b*) Transverse conduction moderate: sequence of response positive, neutral, and negative, *e.g.*, main pulvinus of *Mimosa.*

(*c*) Pulvinus thin and transverse conduction pronounced: transient and hitherto unnoticed positive followed by predominant negative; application of stimulus on the opposite and more excitable side produces movement in the same direction, now positive response. The result would thus appear to be independent of the direction of light. Examples are found in the photonastic movements of lateral leaflets of *Mimosa pudica* and leaflets of *Biophytum sensitivum.*

The following is a diagrammatic representation of the typical cases:—

(Arrow represents direction of incident light.)



(*a*) Radial thick organ. Transverse conduction absent. Response positive.

(*b*) Radial thin organ. Presence of dots represents possibility of transverse conduction. Sequence of response: positive, neutral, and negative.

(*c*) Anisotropic thick organ. Thick line represents the more excitable opposite side. Sequence of response: positive, neutral, and pronounced negative.

(*d*) Anisotropic thin organ. High transverse conductivity. Sequence of response: positive, quickly masked by negative.

When light strikes on the opposite side, the sign of response in (*a*) and (*b*) will remain unchanged. In (*c*) and (*d*) the effect will be only positive.

The demonstration of these different growth-reactions is given in the following series of experiments.

15. *Dia-phototropism and Negative Phototropism.*

Oltmanns found that the seedling of *Lepidium sativum* assumed a transverse or dia-heliotropic position under intense and long-continued action of light of 600,000 Hefner lamps. He regards the transverse as the indifferent position. In reality it is the expression of balanced neutralisation caused by transversely conducted excitation. I obtained such neutralisation with prolonged unilateral exposure to arc-light. The first effect was positive. This was gradually neutralised under continued exposure for two hours; even then the neutralisation was not complete.

Expt. 13.—From the theoretical considerations that have been advanced it would appear that a reversal can only take place under intense light and where there is a possibility of conduction in a transverse direction. Acting on this idea, I employed light from a mercury-vapour lamp, which emits the most effective violet and ultra-violet rays. The specimen was a thin seedling of the rice plant, *Oryza sativa.* The first effect was a positive curvature; this was neutralised and reversed with increasing transverse conduction. The neutralisation was completed in six minutes. The response was afterwards reversed to pronounced negative* by the continued action of light (fig. 15, *a*).

There is a difficulty in connection with the reversal of response which cannot be explained by mere conduction of excitation to the opposite side, for in a radial organ the contraction of the distal side due to transverse conduction of excitation cannot be greater than that of the directly excited proximal side. The only explanation that remains is that a relaxation takes place on the proximal side by the direct action of intense and long-continued stimulation. The following experiment was undertaken to decide the question.

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* For explanation of negative phototropism of certain roots *cf.* 'Plant Response,' p. 601.

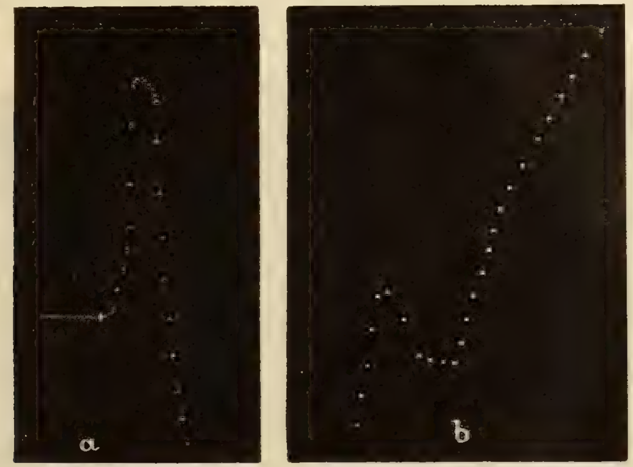

FIG. 15.—*a*, positive, dia-, and negative phototropic response of *Oryza* under continued unilateral stimulus of intense light from arc-lamp ; *b*, record on moving plate of modification of growth under continued stimulation of ultra-violet from mercury-vapour lamp. Application of light at thick dot. Note arrest of growth, contraction and subsequent renewal of growth.

Expt. 14. *Reversal of normal Retardation of Growth under prolonged Stimulation.*—I took a seedling of *Oryza* and by means of inclined mirrors applied light from a mercury-vapour lamp to act on all sides of the organ. The record (fig. 15, *b*) shows that after the normal contraction and retardation a reversal took place, resulting in expansion. The result is not unlike the contraction of muscle passing into relaxation under continuous stimulation. The first effect of stimulation was an arrest of growth and pronounced contraction, but afterwards growth became renewed. It will thus be seen that while under normal circumstances growth is retarded or arrested by light, under prolonged illumination the organ may sometimes recover itself from the state of arrested growth.

16. *Radio-thermotropism.*

Expt. 15.—I have explained (Section 13) that rise of temperature and radiation of heat induce opposite physiological effects. In the thermonastic reaction of the Crocus flower, rise of temperature induces an acceleration of growth, but radiant heat causes a retardation (*cf.* Expt. 10). The tropic effect of thermal rays is as strong, if not stronger, than that induced by the effective blue rays in the spectrum. This will be seen in the following record, obtained in the stem of *Dregea volubilis,* where successive short exposures to thermal rays from a platinum spiral gave positive responses followed by recovery. Continuous exposure gave all the different phases of maximum positive, neutralisation, and reversal into negative (fig. 16), just as under violet and ultra-violet rays from the mercury lamp. The reversal into negative took place within the comparatively short period of 7 minutes. It will thus be seen that, in inducing phototropic curvature, the heat-rays in sunlight play as important a part as the more refrangible rays of the spectrum.

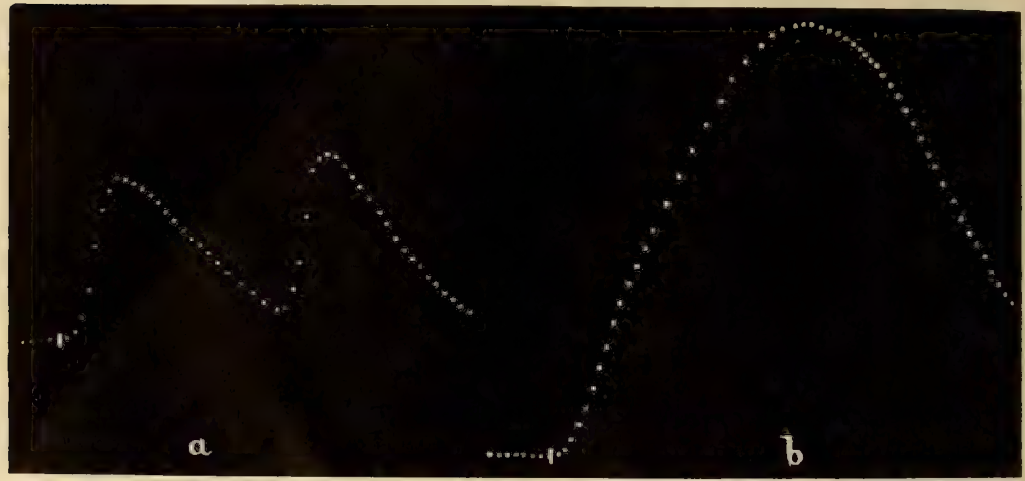

Fig. 16.—*a*, positive responses to short exposure to thermal radiation; *b*, positive, neutral, and reversed negative under continued action of radiation. The negative response went off the plate. Successive dots at intervals of 5 seconds.

17. *Photonasty.*

The leaflets of *Mimosa pudica,* as has been stated before, fold themselves upwards when strongly illuminated either from above or below. Under similar circumstances the leaflets of *Biophytum sensitivum* and *Averrhoa* fold downwards. Diffuse stimulation by electric shock brings about upward closure of leaflets of *Mimosa,* and downward closure of those of *Biophytum* and *Averrhoa.* The excitability of the pulvinule is thus greater on the upper side in *Mimosa* and on the lower side of *Biophytum* and *Averrhoa.*

Expt. 16.—I constructed a very delicate lever, with very little weight, in order to obtain a record of the response of the minute leaflets. Light of moderate intensity from an incandescent lamp was applied on the less excitable side of the pulvinule, that is to say, on the lower side of *Mimosa* and the upper side of *Averrhoa* leaflets. The results show that the immediate response was in both cases positively phototropic, *i.e.*, a movement towards the source of illumination. This effect was subsequently reversed to strong negative by transverse conduction (fig. 17). Had a delicate means of record not been available, the gradual transition from positive to negative phototropic curvature would have passed unnoticed. A continuity is thus established between tropic and nastic reactions, rendering the assumption of specific sensibility for each class of phenomena quite unnecessary.

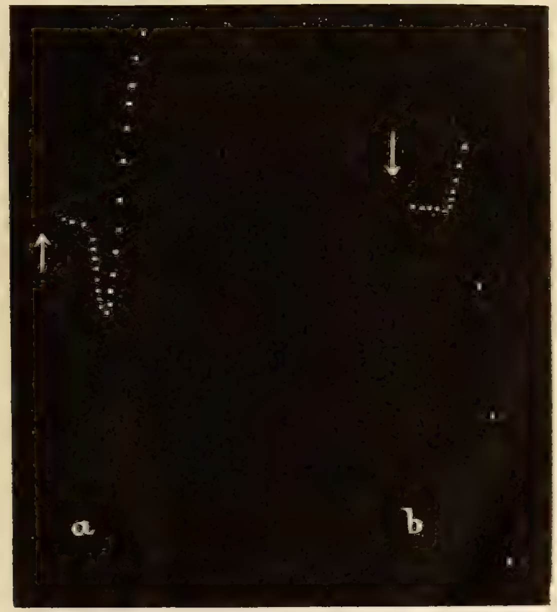

FIG. 17.—Photonastic response : *a*, effect of light applied below on *Mimosa* leaflet; preliminary down or positive movement followed by strong negative up movement. *b*, effect of light applied above on *Averrhoa* leaflet; preliminary up or positive movement followed by pronounced down or negative movement. It is to be noted that movement towards source of illumination is positive. Later and more pronounced negative movement is due to transmitted excitation to the more excitable half of the pulvinule. (Up-movement represented by up-curve and *vice versâ.*)

The following laws express the movements of plants under external stimuli and are of universal application :—

1. All forms of stimuli induce contraction as their direct and expansion as their indirect effect.
2. Unilateral stimulation causes positive curvature by contraction of the proximal and expansion of the opposite side.
3. Transverse conduction of excitation induces contraction of opposite side, neutralising or reversing the positive responsive curvature.

4. These effects are accentuated by the differential excitabilities of the two halves of an anisotropic organ.

D. RELATION BETWEEN RESPONSE TO STIMULATION OF GROWING AND OF NON-GROWING ORGANS.

18. *Similarity of Response of Growing and of Non-growing Organs.*

I have throughout this paper adduced numerous instances of the essential similarity between the response of motile and that of growing organs. The following tabular statement will show that a fundamental reaction finds expression in diverse ways, according to different methods of recording response in motile, non-motile, and growing organs :—

Table IV.—Mechanical and Electrical Responses in Motile, Non-motile and Growing Organs.

Inducing cause.	Change of form.	Mechanical response.	Electrical response.
Direct stimulation	Contraction	Fall of leaf ; negative response	Negative response.
Indirect stimulation	Expansion	Erection of leaf ; positive response	Positive response.
Effect of increasing turgor	Expansion	Erection of leaf ; positive response	Positive response.

It has been shown that any agent which increases the excitability of pulvinated organs, as measured by the amplitude of response, mechanical or electrical, likewise induces a positive variation or enhancement of the rate of growth. Conversely, a depressor which brings about a diminution in the amplitude of their mechanical and electrical response also causes depression in the normal rate of growth.

The tropic effect of light is the same on growing as on motile organs. The particular rays which are effective in one case are also effective in the other. Red and yellow rays are ineffective in both. In one case, as in others, we obtain the positive, the dia-positive, and the negative. The tropic movements are determined in both by the same combination of the effects of direct and indirect stimulation.

Owing to the varying combinations of effects of numerous unknown factors the phenomenon of growth presents many perplexities. We may take, for example, the case of the action of external stimulus on growth. Here subminimal stimulus induces one effect and moderate stimulus the very opposite.

Should the tonic condition of the plant happen to be below par, the effect of stimulus will be an abnormal acceleration of growth, but during the course of the experiment (owing to the continued action of stimulus) the effect will mysteriously revert to the normal retardation. The point of application of stimulus will introduce further complication, indirect stimulation inducing an effect precisely the opposite to that of direct application. The response under unilateral stimulation is further modified by transverse conductivity, by the intensity of stimulation, and the differential excitability of the organ. In an actual experiment the permutation and combination of these different factors will give rise to effects which will, no doubt, appear as highly capricious. These complexities have led Pfeffer to state that an empirical treatment of the subject of growth is all that is possible in the present state of our knowledge. He, however, adds that "deductive treatment still remains the ideal of physiology, and only when this ideal has been attained shall we be able to obtain a comprehensive view of the interacting factors at work in the living organism."*

I have attempted in the present paper to contribute towards a deductive treatment of the subject by investigating the isolated effect of each of the numerous complicating factors.

Among these, we have the changing influence of the environment, which cannot be kept strictly constant for more than a short time. Besides this, "there are the numerous and varied stimulating and mechanical interactions between different organs." The changing influence of the environment can in practice be eliminated by reducing the period of experiment to a very short time (rendered possible by the employment of the high magnification crescograph) and studying the influence of one factor at a time. Shortening the period of experiment also excludes the interaction of distant organs, for its influence can only be exerted after a certain lapse of time. The organ to be experimented on can thus be isolated from the influence of changing environment and from the interactions of neighbouring organs. In this state of isolation the response under normal conditions is found to be very definite. A given modification of normal response can, moreover, be traced to the definite variation of effect due to the change in the intensity and point of application of stimulus, or in the tonic condition of the reacting organ.

SUMMARY.

The most important results that I have so far obtained from experiments with the crescograph are briefly as follow:—

1. Under lowering of temperature the growth-rate undergoes a diminution

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* Pfeffer, *ibid.*, vol. 2, p. 1.

and arrest at a definite temperature. In *Scirpus Kysoor* the arrest takes place at 22° C. Rise of temperature enhances the rate of growth to an optimum, beyond which there is a decline. At 60° C. a violent contraction takes place, which is the spasm of death.

2. Heat induces two effects, which are diametrically opposite to each other. The thermal effect of heat is to increase the rate of growth; the radiation effect is a retardation.

3. The closest parallelism has been established between the response to stimulation given by pulvinated and by growing organs respectively. Conditions which give rise to negative mechanical or electric response of the former also give rise to negative variation or retardation of growth. This is also true of positive mechanical or electric response and positive variation or enhancement of growth. The physiological machinery is the same in pulvinated and non-pulvinated, in growing and non-growing organs.

4. Every stimulus is shown to give rise to two distinct impulses: a hydro-positive, independent of the conductivity of the tissue, and an excitatory negative, which is dependent on the conducting power. The former is transmitted quickly; the latter, being a phenomenon of conduction of protoplasmic change, is propagated slowly. The hydro-positive impulse gives rise to expansion, the excitatory negative to contraction. The transverse conductivity of an organ is very much less than its longitudinal conductivity.

5. Investigation on the effect induced by all forms of stimuli has led to the establishment of the following law: *Direct application of stimulus induces contraction; indirect application gives rise to expansion.*

Direct stimulation of the responding region causes a contractile fall of the motile leaf, or a retardation of growth in a growing organ. The transmitted or indirect effect of stimulus applied at a distance is to induce an erection of the leaf and an acceleration of the rate of growth.

6. This latter fact may offer an explanation of the opposite effects of the stimulus of gravity on root and shoot. In the root, the stimulus is received at the tip, and transmitted to the distant region of growth. Stimulation here is indirect. In the shoot, stimulation is direct, since the growing region of the shoot is both sensitive and responsive. The opposite signs of response in shoot and root may, therefore, be due to the fact that in one case the stimulation is direct and in the other indirect.

7. Tropic movements also are subject to the laws of direct and indirect stimulation. The directly excited proximal side undergoes contraction, the opposite side undergoes expansion; both these factors conspire to induce a positive curvature. This fundamental effect undergoes modification on account of transverse conduction of excitation bringing about neutralisation

or reversal. This may become accentuated by differential excitability of the two sides of the organ.

8. The normal effect undergoes modification when the tissue is in the condition of sub-tonicity. The effect of stimulus on a sub-tonic pulvinus is a positive mechanical or positive electrical response; corresponding to this is the positive variation or acceleration of growth in sub-tonic tissues, under the stimulus of light or of electric shock. Continuous stimulation converts abnormal positive to normal negative.

9. Mechanical friction induces retardation of growth; wounds cause a more pronounced and persistent retardation. Unilateral stimulation of contact brings about positive curvature in a tendril by the retardation of growth at the proximal and acceleration at the opposite side.

10. Electric stimulus induces retardation. Growth is affected by an intensity of electric shock which is far below human perception; the latent period diminishes with increasing intensity of stimulus from 6 seconds to less than a second. The incipient contraction under feeble stimulus culminates under strong stimulus to a marked contraction of the growing organ. The response of the growing organ is similar to the response of pulvinated organs. In the polar action of electric current on growth, the anode is found to enhance and the cathode to depress the normal rate. Unilateral stimulation causes a positive electrotropic curvature.

11. Light causes retardation of growth; intense illumination arrests growth; but under long-continued exposure, growth may become renewed. Unilateral application of increasing intensity causes a positive, dia-phototropic, and reversed negative response. The more refrangible rays are the more effective, the ultra-violet being most so. The thermal rays in the infra-red are also effective. The phototropic curvature under sunlight is partially due to the obscure radiation.

12. These phototropic effects are accentuated in anisotropic organs where one side is more excitable than the other. Tropic and nastic movements are not distinct phenomena, but a continuity exists between the two.

13. From the above it will appear that the generalisation has been reached—that all the induced movements of plants under environmental changes are the definite effects of direct and indirect stimulation.

I take this opportunity to express my acknowledgment to Mr. P. C. Lyon, C.S.I., the retired Minister in charge of Education to the Government of Bengal, for the facilities afforded to me and for his unremitting interest in research.

I also wish to offer my special thanks to the Royal Society which has not

only extended the hospitality of publication to me for the last 22 years, but has also afforded me assistance from the Government Grant placed at its disposal.

It is now my good fortune to have been able to found a Research Institute, from which the present paper forms the first instalment of work. It is my wish that the facilities of this Institute should be available to research workers from all countries. In this I am attempting to carry out the tradition of my country, which, so far back as 25 centuries ago, welcomed scholars from all parts of the world within the precincts of its ancient seats of learning at Nalanda and at Taxilla.

Investigations dealing with the State of Aggregation. Part IV.—*The Flocculation of Colloids by Salts containing Univalent Organic Ions.*

By S. B. Schryver, D.Sc., and Nita E. Speer.

(Communicated by V. H. Blackman, F.R.S. Received June 18, 1918.)

(From the Department of Plant Physiology and Pathology, Imperial College of Science and Technology.)

Theory and Scope of the Researches.

In an earlier paper* the action of salts in a heterogeneous system was discussed, and the factors which exert influence on the state of hydration and aggregation of the "colloid phase" were summarised. The present communication deals with only one of these factors, viz., the surface-tension of the solutions and the relationship between this property and the capacity of salts for flocculating colloids.

The mechanism by means of which this flocculation is produced is still a matter of considerable controversy. The view which appears, up to the present, to be most widely maintained is that the precipitation depends, in the first instance, on the adsorption of the active (precipitating) ion by the colloid. According to Freundlich, who is mainly responsible for what may be termed the adsorption hypothesis,† the action of the ions can be explained in the following manner: Before coagulation takes place, the colloidal particles must lose a certain part of their charge, so that the difference of potential between colloid and dispersion medium does not exceed a certain critical maximum (Powis[*]). This partial discharge can be brought about, when the precipitating capacity of various salts is considered, by the adsorption of equivalent quantities of ions with a charge opposite to that of the colloids; if one particular active ion is more readily adsorbed by a colloid than another of equal valency, a salt containing the former ion will produce the degree of discharge necessary for flocculation in a lower concentration of solution than will the salt which contains the ion that is less readily adsorbed. By means of the adsorption hypothesis, Freundlich has endeavoured to explain the numerical relationship between the flocculating capacities of salts containing ions of different valencies.[†] The greater precipitating capacity of salts containing organic ions as compared with those containing inorganic ions has also been explained by the assumption that the former are more readily adsorbed than the latter. Furthermore, typical adsorption isotherms have been obtained, when the distribution of salts between colloid and dispersion medium has been determined.[‡]

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* Schryver and Hewlett, 'Roy. Soc. Proc.,' B, vol. 89, p. 361 (1916). For Parts I–III of this series see 'Roy. Soc. Proc.,' B, vol. 83, pp. 96, 113, 119 (1910).

† Freundlich, 'Zeitsch. Physikal. Chem.,' vol. 73, p. 385 (1910); Freundlich and Gann, 'Kolloid-chem. Beihefte,' vol. 8, p. 108 (1916).

A totally different conception has been put forward by Duclaux,[§] who regards the colloid particle as a micella containing a "granule" of the colloid proper associated with "active" ions derived from a substance used in the preparation of a colloidal solution. Thus, in the case of the ferric hydroxide sol, prepared by the dialysis of a solution of ferric hydroxide in ferric chloride, the granules consist of ferric hydroxide, and they are associated with active chlorine ions, and flocculation on addition of a salt is regarded as taking place by the substitution of the active ions by equivalent quantities of others by a purely chemical reaction. It is not necessary to discuss in detail Duclaux's hypothesis, as certain of his assumptions are no longer tenable, as, for example, those dealing with the osmotic pressure of colloidal solutions, which are not in accord with subsequent researches of Perrin on the equipartition of energy in solutions.

More recently still, Pauli and Matula[||] have put forward a somewhat different hypothesis, according to which flocculation is regarded as due mainly to a chemical action. Their conceptions are founded on a study of the ferric hydroxide sols, which they regard as complex salts; that prepared from ferric chloride solutions they conceive as being built up according to the formula

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[*] 'Zeitsch. Physikal. Chem.,' vol. 89, p. 195 (1914).

[†] Freundlich, 'Kapillarchemie,' Leipzig, 1909, pp. 354–355.

[‡] See *e.g.* Maffia, 'Kolloid-chem. Beihefte,' vol. 3, p. 85 (1911), and Gann, *ibid.*, vol. 8, p. 63 (1916).

[§] 'Journ. Chim. Physique,' vol. 5, p. 29 (1907), and vol. 7, p. 405 (1909).

[||] 'Kolloid Zeitsch.,' vol. 21, 49 (1917).

$$x\mathrm{Fe(OH)_3} \,.\, y\mathrm{Fe}^{\cdots} \,\ldots\ldots\ldots\, 3y\mathrm{Cl}',$$

and as being a moderately strong electrolyte. On addition of a salt containing an anion in common, *e.g.*, sodium chloride, the dissociation of the colloid is depressed. The coagulating power of other electrolytes appears to be determined by the solubility product of the colloid ion and the anion of the coagulant. The depression of the dissociation is associated with instability of the sol. The conceptions of Pauli and Matula are founded mainly on what appear to be careful electrometric measurements of chlorine and hydrogen ion concentration, but it must be urged in criticism of these, that their value is uncertain when applied to what are undoubtedly heterogeneous systems.

Now if the flocculation of colloids by salts is due, in the first instance, to an adsorption process, some relationship between the flocculation concentration and the surface tension of the salt solutions should be expected; the lower the surface tensions of the solutions, the greater should be the precipitating power. If, on the other hand, a double decomposition involving the "active" ion of the colloid and the corresponding ion of the precipitant, as postulated in the conceptions of Pauli and Matula and of Duclaux, is an essential feature, no such relationship should exist. In the latter case, furthermore, the relative precipitating capacity of salts belonging to a series should vary from colloid to colloid.

To investigate the relationship between surface tension of salt solutions and their capacity for flocculating colloids, salts containing organic ions were chosen, as their normal solutions display wide variations in the first-named property. A certain number of isolated experiments on flocculation by such salts have been published in the literature, but no systematic examination on a wide basis has been recorded.*

For the present work, a series of sodium salts was selected in the first instance, which had been previously employed in determining the relationship between surface tension and disaggregating power. This included the following, which are placed in order of diminishing surface tension of their normal solutions: Formate > acetate > lactate > monochloracetate > dichloracetate > trichloracetate > salicylate > benzoate. The disaggregating action of these salts shows a close relationship with the surface tension of their solutions, those with the lowest surface tensions exhibiting the greatest disaggregating action.*

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* See *e.g.*, Freundlich, 'Zeitsch. Physikal. Chem.,' vol. 44, p. 144 (1903); Picton and Linder, 'Trans. Chem. Soc.,' vol. 87, pp. 1922 (1905); Traube and Onodera, 'International. Zeitsch. für Physik.-chem. Biologie,' vol. 1 (1914).

The disaggregating power has been determined in the following instances: (1) As regards their power of dispersing the globulins (this series, Part I). (2) Their action on inhibiting the formation of methylene iminopeptones (this series, Part II). (3) Their influence on the critical solution temperatures of phenol and water, and their effect on the solubility of certain salts in water (this series, Part III).† In the form of calcium salts these acids also exhibit an inhibiting action on the formation of the cholate gel when the surface tensions of the solutions is lower than that of pure water; in this case, the greater the concentration, the greater would be the time required for clot formation if the lowering of surface tension impeded aggregation; the increase in the concentration of the calcium ions, however, diminishes the time, the calcium and anions acting antibatically. In spite of this fact, however, the general relationship between surface tension and disaggregating capacity is clear from the experiments.‡

The above mentioned salts form, therefore, a perfectly well-defined "series" as regards their disaggregating capacity, and it was of special interest to ascertain whether their power for flocculating colloids ran in any parallel with this action. One other sodium salt was added to the number investigated, viz., that of benzene sulphonic acid. This belongs to a class of salts exhibiting, what Neuberg terms "hydrotropic" phenomena,§ that is, the capacity of rendering certain substances soluble in water which are only very slightly soluble in the absence of salts. A similar phenomenon was recorded by one of the authors (see this series, Part III), and ascribed by him to the effect of the salt in lowering the surface tension of the aqueous solution and thus markedly increasing the disaggregating power of the solvent. It is recorded in the sequel that the normal solution of sodium benzene sulphonate has a very low surface tension. Benzene sulphonic acid is, furthermore, a strong acid as compared with salicylic acid, and, for this reason alone, the addition of its sodium salt to the list of those investigated was desirable.

As both negative and positive sols were included in these researches, and as the former are more sensitive to the action of the cations and the latter to the anions, it was necessary to investigate in addition the influence of the surface tension of solutions of a series of salts containing a common anion. For this purpose the hydrochlorides of organic bases were chosen. As many of these have only a low basic dissociation constant, their hydrochlorides undergo an appreciable amount of hydrolytic dissociation in water, and this introduces a complication which it is desirable to avoid. For this reason, only those bases are included within the sphere of investigation, of which the basic dissociation constants are greater than that of ammonia.*

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* There is very little difference between the surface tensions of the normal benzoate and salicylate solutions. The latter, which has a slightly greater surface tension in normal solution, has a greater disaggregating power, which may be ascribed to the differences in the viscosity. For discussion on these points, see Part I of this series.

† 'Roy. Soc. Proc.,' B, vol. 83, pp. 96, 113, 119 (1910).

‡ Schryver, 'Roy. Soc. Proc.,' B, vol. 87, p. 366 (1914).

§ 'Biochem. Zeitsch.,' vol. 76, p. 107 (1916).

Before proceeding to discuss the results obtained, it is necessary to refer to another possible influence of the action of the surface tension of solutions on the process of aggregation of colloids. If adsorption is the main factor in production of the flocculation, it would follow that the lower the surface tension the smaller the concentration of the salt solution necessary to produce the requisite amount of discharge and consequent aggregation. The lowering of the surface tension at the interface colloid-dispersion medium might conceivably tend to act against aggregation, so that two possible actions might be ascribed to the surface tension effect, which are antibatic. Zsigmondy, however, in a very recent paper,† has expressed the opinion that surface tension effects play but little part in the aggregation in the case, at any rate, of the suspensoid colloids, and that the process takes place by direct attraction of the colloids particles as soon as the potential difference between these and the dispersion medium has been reduced below a certain critical maximum.‡ The dynamics of aggregation has been mathematically treated on this assumption by the late E. von Smoluchowski, and forms the subject of one of the last papers published by him before his death.§ Under any circumstances, the surface tension at the interface suspensoid colloid-dispersion medium is probably high, and the relative amount of lowering produced by the addition of the quantity of salt necessary to bring about aggregation is so small that the mechanical effect of surface tension acting against flocculation is probably quite negligible. This supposition is borne out by experiments recorded in the sequel.

As a general result of these investigations, it was found that there is generally no relationship between the surface tensions of solutions of salts and their flocculating capacity. In the case of positive sols, wide variations were found in the precipitating capacity of the sodium salts, as in these cases the active ion is the cation; on the other hand, the precipitating capacity of the hydrochlorides of bases varied only within narrow limits in spite of the wide variations in the surface tension of the normal solutions. In the case of the negative sols wide variations were observed in the action of the hydrochlorides but not in the case of sodium salts. The ferric hydroxide sol was flocculated more readily by the sodium salts of weak acids than by the corresponding salts of strong acids, and the same fact holds, though with some exceptions, to the action of these salts on other sols prepared by the dialysis of salts of inorganic acids. These facts might suggest that in the case of the ferric hydroxide sol the colloidal phase might consist of a heavily hydrated ferric hydroxide (*cf.* researches of van Bemmelen) holding ferric chloride, water, and hydrochloric acid in chemical equilibrium with one another :—

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* Reference was made to Lundén's tables for this constant; no record was found of K_b for hexylamine. From analogy one would judge that it is of the same order as that of ammonia.

† 'Zeitsch. Physikal. Chem.,' vol. 92, p. 600 (1918).

‡ 'Zeitsch. Physikal. Chem.,' vol. 92, p. 129 (1917).

§ Powis, *loc. cit.*

$$FeCl_3 + 3H_2O \rightleftarrows Fe(OH)_3 + 3HCl,$$

and that the sodium salt of a weak acid would react with the hydrochloric acid with the formation of sodium chloride and a free slightly dissociated organic acid and thus lead to the discharge of the colloid phase. Until more is known, however, as to the distribution of electrolytes between a hydrated colloid phase and the dispersion medium, a problem which involves the consideration of several factors (see Schryver and Hewlett *loc. cit.*), discussion on this matter is of little value. The results found generally do not support the adsorption hypothesis of the flocculating action.

In only one case, and the exception appears to be an important one, is there any marked relationship between the surface tension of the salt solution and the flocculating capacity of the salts, and it is that of the action of the hydrochlorides on the flocculation of mastic. In this case the salts giving solutions with the lower surface tensions have the greater precipitating power. Only one salt, viz., tetraethylammonium chloride falls out of the series and this is different chemically from the hydrochlorides.

This fact seems to suggest that a differentiation may be made between the various suspensoids colloids which takes into account the origin of the electrical charge. In the case of a sol such as ferric hydroxide, the stability depends upon the association of the sol with "active" ions, derived from the salt from which the colloid is prepared (Cl' ions when ferric hydroxide sol is prepared from ferric chloride, and NO_3' ions when prepared from the nitrate). As the ions are gradually removed by hydrolysis, the stability of the sol decreases. Aggregation resulting finally in flocculation is here accompanied by a marked change in the components of the colloid phase. But it is conceivable that sols can exist in which the charge is due to ions belonging to the molecule of the substance itself, which is the main or perhaps the only body, apart from water of hydration, in the colloid phase. The colloid may be, *e.g.*, an acid substance containing carboxyl groups which will dissociate into readily diffusible hydrogen ions and large slightly diffusible cations. The former would then exist in the outer layers of the colloid phase, and be held electrostatically to the slowly moving cation in the inside. Mastic (a resin acid) is probably the only substance investigated which would form a sol of this class, and it is noteworthy that in this case only is there a close relationship between the surface tensions of their solutions and the flocculating capacity of salts. It is proposed to designate colloids of this class as *endionic* and those of the other class *exionic*. It is hoped, at a more favourable opportunity, that these investigations may be extended to other substances, such as the proteins, which probably belong to this class. Pressure of other work and difficulty in obtaining the requisite materials are, for the moment, obstacles to such an extension.

Surface Tensions of Normal Solutions.

The materials employed were, when obtainable, Kahlbaum's preparations. The dichloracetic acid was prepared in the laboratory from chloral-hydrate by Wallach's method. The lactic acid was obtained from a crude commercial specimen by recrystallisation of the calcium salt and its subsequent decomposition by anhydrous oxalic acid (in slight deficiency) in pure ether. Mono- and trimethylamine were obtained in a satisfactory state of purity as hydrochlorides by a method recently described by Werner.* The corresponding salt of dimethylamine, in spite of several attempts, could not be obtained pure by Werner's method, and the base was finally prepared by the action of sodium hydroxide on dimethylaniline. The authors are indebted to Prof. Philip for a sample of triethylamine and tetraethylammonium bromide, from which latter substance the chloride was prepared in the usual manner. The surface tensions of the sodium salts are quoted from the first paper of this series, with the exception of that of benzene sulphonic acid. The surface tensions of this and the other substances in the Table were determined by the drop method with the use of a Traube stalagmometer. The specific gravities were determined in a small pycnometer. The sodium salt solutions were made by the method described in the first paper. The chloride solutions were standardised by silver nitrate. The usual precautions for obtaining sufficiently pure water were taken.

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* 'Trans. Chem. Soc.,' vol. 111, p. 844 (1917).

Surface Tensions of Normal Salt Solutions. $\gamma_{H_2O} = 1$.

	Sodium Salts.			(Hydro) Chlorides.	
1	Sodium chloride	1·032	I	Sodium chloride	1·032
2	Sodium formate	1·020	II	Ammonium chloride	1·027
3	Sodium lactate	1·013	III	Monomethylamine hydrochloride	1·017
4	Sodium acetate	1·004	IV	Trimethylamine hydrochloride	1·0074
5	Sodium monochloracetate	1·002	V	Dimethylamine hydrochloride	1·006
6	Sodium dichloracetate	0·970	VI	Tetraethylammonium chloride	0·965
7	Sodium trichloracetate	0·905	VII	Piperidine hydrochloride	0·953
8	Sodium salicylate	0·902	VIII	Triethylamine hydrochloride	0·950
9	Sodium benzoate	0·897	IX	Benzylamine hydrochloride	0·878
10	Sodium benzene sulphonate	0·816	X	Isoamylamine hydrochloride	0·728
			XI	Hexylamine hydrochloride	0·516

Stalagmometer and pycnometer readings were taken at 15° C.

Flocculation Capacity of Salts.

Attempts were made to carry out the precipitation under as nearly as possible constant conditions. To 2 c.c. of the sol in a series of small test-tubes were added 2 c.c. of various dilutions of the salt in concentrations diminishing in geometrical ratio. The salt solution was rapidly poured into the sol and the mixture was then poured back into the first test-tube. The limits between which precipitation took place were then noted after several hours. The finer limits were then determined by carrying out the precipitation with a number of dilutions between the coarser limits, and the second set of experiments were carried out at constant temperature (20°), the salt solutions and sols being kept at this temperature before mixing. The observations were generally made after an interval of 17 hours. In the following Tables the numbers refer to the dilutions of the salts (after mixing with the sols) in millimols per litre. + + + indicates complete precipitation, + + not quite complete precipitation, + slight turbidity, 0 no change in sol visible to naked eye. In certain cases the finer limits were not determined, especially when these were high, as larger amounts of costly materials necessary for the experiment were not available.

Ferric Hydroxide.

Prepared by dilution of *liquor ferri dialysat.* B.P., diluted with seven times the volume of water. 100 c.c. contained 1·33 per cent. solid, dried at 100° C.

Sodium Salts.

					0.
Formate	12·5 + + +	10·93 + +	9·37 + +	7·82 +	7·03
Lactate	7·82 + + +	7·03 + +	6·25 + +	5·64 +	4·60
Acetate	5·46 + + +	4·60 + +	3·90 +		3·51
Monochloracetate	18·75 + + +	15·62 + +	14·06 + +	12·50 +	10·93
Dichloracetate	56·25 + + +	50·0 + +	43·75 +		37·50
Trichloracetate	56·25 + + +	50·0 + +	43·75 + +	37·5 +	31·25
Salicylate	2·69 + + +	2·30 + +			1·95
Benzoate	3·51 + + +	3·12 + +	2·69 +		2·30
Benzene sulphonate	21·87 + + +	18·75 + +			15·62

(Hydro) Chlorides.

		0.
Sodium	250 + + +	125
Ammonium	250 + + +	125
Monomethylamine	250 + + +	125
Trimethylamine	250 + + +	125
Dimethylamine	250 + + +	125
Tetraethylammonium	125 + + +	112·5
Piperidine	250 + + +	125
Triethylamine	250 + + +	125
Benzylamine	250 + + +	125
Isoamylamine	250 + + +	125
Hexylamine	250 + + +	125

Zirconium Hydroxide.

Prepared by repeated evaporation of Zirconium nitrate solution by Müller's* method. 100 c.c. contained 1·93 per cent. solid, dried at 100° C.

Sodium Salts.

							0.
Formate	56·25 + + +	50·0 + +	43·75 +	37·5 +			31·5
Lactate	—						>500
Acetate	62·5 + + +	56·25 + +	50·0 + +	43·75 +	37·5 +		31·25
Monochloracetate	56·25 + + +	50·0 + +	43·75 + +	37·5 +			31·25
Dichloracetate	50·0 + + +	43·75 + +					37·50
Trichloracetate	43·75 + + +	37·5 + +					31·25
Salicylate	14·06 + + +	12·5 + +	9·37 + +	7·82 +	7·63 +		6·25
Benzoate	12·5 + + +	10·93 + +	9·37 + +	7·82 +	7·03 +		9·25
Benzene sulphonate	225 + + +	200 + +	175 + +	150 +	125 +	112·5 +	100·0

(Hydro) Chlorides.

No flocculation produced at a strength of 500 millimols per litre.

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* 'Zeitsch. Anorg. Chem.,' vol. 52, p. 316 (1907).

Cerium Hydroxide.

Prepared by dialysis of ceric ammonium nitrate by Biltz's method.* 100 c.c. contained 0·049 per cent. solid, dried at 100° C.

Sodium Salts.

		0.
Formate	0·46+ + + 0·38+	0·31
Lactate	0·23+ + + 0·19+ + 0·17+ + 0·15+	0·13
Acetate	0·46+ + + 0·38+ + 0·35+	0·31
Monochloracetate	0·53+ + + 0·46+ + 0·38+ + 0·35+ +	0·31
Dichloracetate	1·87+ + + 1·56+ + 1·40+ + 1·25+	1·09
Trichloracetate	1·87+ + + 1·56+ + 1·40+	1·25
Salicylate	0·19+ + + 0·17+ + 0·15+ + 0·13+	0·11
Benzoate	0·23+ + + 0·19+ + 0·17+ + 0·15+ 0·13+	0·11
Benzene sulphonate	1·25+ + + 1·09+ +	0·93

(Hydro) Chlorides.

		0.
Sodium	3·75+ + + 3·18+ + 2·81+ + 2·43+ + 2·18+ 1·87+	1·56
Ammonium	3·75+ + + 3·18+ + 2·81+ + 2·43+	2·18
Monomethylamine	4·37+ + + 3·75+ + 3·18+ + 2·43+ + 2·18+ 1·87+	1·56
Trimethylamine	2·81+ + + 2·43+ + 2·18+ + 1·87+ + 1·56+	1·40
Dimethylamine	3·75+ + + 3·18+ + 2·18+ + 2·43+ + 2·18+ + 1·87+	1·56
Tetraethylammonium	2·43+ + + 2·18+ + 1·87+ + 1·56+ + 1·40+ 1·25+	1·09
Piperidine	3·75+ + + 3·18+ + 2·81+ + 2·43+ 2·48+	1·87
Triethylamine	5·63+ + + 5·0 + + 4·37+ + 3·75+ 3·18+ 2·81+ 2·43+ 2·18+	1·87
Benzylamine	3·75+ + + 3·18+ + 3·81+ + 2·43+	2·18
Isoamylamine	4·37+ + + 3·75+ + 3·18+ 2·81+ 2·43+ 2·18	1·87
Hexylamine	3·18+ + + 2·81+ + 2·43+ + 2·81+	1·56

Victoria Blue B. (*Badische Company.*)

Solution dialysed for four days. 100 c.c. contained 0·094 solid, dried at 100° C.

Sodium Salts.

		0.
Formate	112·5 + + + 100 +	87·5
Lactate	225 + + + 200 + + 175 +	150
Acetate	125 + + 112·5 +	100
Monochloracetate	56·15+ + + 50·0 + + 43·75+ + 37·5 +	31·25
Dichloracetate	14·06+ + + 12·5 + + 10·93+ + 9·37+ 7·82+ +	7·03
Trichloracetate	1·95+ + 1·+36	0·97
Salicylate	0·97+ + 0·68+	0·48
Benzoate	14·06+ + + 12·5 + + 10·93+ 7·82+	7·03
Benzene sulphonate	1·95+	1·36

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* 'Berl. Berichte,' vol. 35, p. 4435 (1902).

(Hydro) Chlorides.

		0.
Sodium	112·5 + + + 100 + + 87·5 + + 75 + +	62·5
Ammonium	112·5 + + + 100 + + 87·5 +	75·0
Monomethylamine	112·5 + + + 100 + + 87·5 + + 75 +	62·5
Trimethylamine	112·5 + + + 100 + + 87·5 + + 75 +	62·5
Dimethylamine	112·5 + + + 100 + + 87·5 + + 75 +	62·5
Tetraethylammonium	21·87 + + + 18·75 + + 15·62 + +	14·06
Piperidine	112·5 + + + 100 + + 87·5 + 75 +	62·5
Triethylamine	112·5 + + + 100 + + 87·5 + 75 +	62·5
Benzylamine	112·5 + + + 100 + + 87·5 + 75 +	62·5
Isoamylamine	112·5 + + + 100 + + 87·5 + 75 +	62·5
Hexylamine	112·5 + + + 100 + + 87·5 + 75 +	62·5

Azo Blue. (Bayer Company.)

Solution dialysed for three days. 100 c.c. contained 0·025 per cent. solid, dried at 100° C.

Sodium Salts.

		0.
Formate	175 + + + 150 + + 125 + + 112 + 100 +	87·5
Lactate	200 + + + 175 + + 150 + + 125 + 112·5 + 100 + 87·5 +	75·0
Acetate	200 + + + 175 + + 150 + + 125 + 112·5 + 100 + 87·5 +	75·0
Monochloracetate	175 + + + 150 + + 125 + + 112·5 + 100 +	87·5
Dichloracetate	200 + + + 175 + + 150 + + 125 + 112·5 +	100
Trichloracetate	150 + + + 125 + + 112·5 + + 100 + + 87·5 +	75·0
Salicylate	200 + + + 175 + + 150 + + 125 + 112·5 + 100 +	87·5
Benzoate	200 + + + 175 + + 150 + + 125 + 112·5 + 100 +	87·5
Benzene sulphonate	175 + + + 150 + + 125 + 112·5 +	100

(Hydro) Chlorides.

		0.
Sodium	200 + + + 175 + + 150 + + 125 + 112 + 100 + 87·5 +	75·0
Ammonium	100 + + + 87·5 + + 75 +	62·5
Monomethylamine	112·5 + + + 110 + + 87·5 + + 75 +	62·5
Trimethylamine	112·5 + + 112·5 + 100 +	87·5
Dimethylamine	125 + + + 112·5 + + 100 + 87·5 +	75·0
Tetraethylammonium	500 +	250·0
Piperidine	87·5 + + + 75 + + 625 + + 56·25 + + 50 + + 43·75 + + 37·5 +	31·25
Triethylamine	250 + + + 225 + + 200 + + 175 + 150 +	125·0
Benzylamine	15·62 + + + 14·06 + + 12·5 + + 10·93 + 9·37 +	7·82
Isoamylamine	21·87 + + + 18·75 + + 15·65 + 14·06 + 12·5 +	10·93
Hexylamine	9·37 + + + 7·82 + + 7·03 + + 6·25 + + 5·46 +	4·60

Brilliant Congo R. (*Berlin Actien-Gesellschaft.*)

100 c.c. contained 0·14 per cent. solid, dried at 100° C.

Sodium Salts.

		0.
Formate	500 + + +	250
Lactate	500 + + +	250
Acetate	500 + + +	250
Monochloracetate	500 + + +	250
Dichloracetate	500 + + +	250
Trichloracetate	500 + + +	250
Salicylate	500 + + +	250
Benzoate	500 + + +	250
Benzene sulphonate	500 + + +	250

(Hydro) Chlorides.

		0.
Sodium	500 + + +	250
Ammonium	—	>500
Monomethylamine	—	>500
Trimethylamine	—	>500
Dimethylamine	—	>500
Tetraethylammonium	—	>500
Piperidine	—	>500
Triethylamine	—	>500
Benzylamine	500 + + + 250 + +	125
Isoamylamine	—	>500
Hexylamine	200 + + + 125 + + 62·5 + +	31·25

Zsigmondy's Scarlet Gold sol.

Prepared by Zsigmondy's method* by reducing gold chloride with formaldehyde.

Sodium Salts.

	Strength in millimols per litre giving change in colour.	r.
Formate	21·87 b, 18·75 m	15·62
Lactate	28·12 b, 25 m, 21·87 m	18·75
Acetate	21·87 b, 18·75 m	15·62
Monochloracetate	25 b, 21·87 m	18·75
Dichloracetate	18·75 b, 15·62 m, 14·06 m	12·5
Trichloracetate	18·75 b, 15·62 m	14·06
Salicylate	25 b, 21·87 m, 18·75 m	15·62
Benzoate	31·25 b, 28·12 m, 25 m, 21·87 m, 18·75 m	15·62
Benzene sulphonate	37·5 b, 31·25 m, 28·12 m	25·0

b = blue; m = mauve; r = red.

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* 'Zeitschr. f. Electro-Chem.,' vol. 4, p. 546 (1898).

Hydrochlorides.

	Strength in millimols per litre giving change in colour.	r.
Sodium	28·12 b, 25·0 m, 21·87 m	18·75
Ammonium	15·62 b, 14·06 m, 12·5 m	10·93
Monomethylamine	15·62 b, 14·06 m	12·50
Trimethylamine	9·37 b, 7·82 m, 7·03 m	6·25
Dimethylamine	15·62 b, 14·06 m	12·50
Tetraethylammonium	10·93 b, 9·37 m, 7·82 m, 7·03 m	6·25
Piperidine	9·37 b, 7·82 m, 7·03 m	6·25
Triethylamine	4·60 b, 3·90 m, 3·51 m	3·12
Benzylamine	3·90 b, 3·51 m	3·12
Isoamylamine	9·37 b, 7·82 m, 7·03 m, 6·25 m	5·46
Hexylamine	3·12 b, 2·69 m, 2·30 m	1·95

b = blue; m = mauve; r = red.

Arsenic Sulphide.

Prepared by the method of Picton and Linder. 100 c.c. contained 0·31 per cent. solid, dried at 100° C.

Sodium Salts.

		0.
Formate	112·5+ + + 100 +	87·5
Lactate	125 + + + 112·5+ + 100 +	87·5
Acetate	125 + + + 112·5+ +	100·0
Monochloracetate	112·5+ + + 100 + + 87·5+ 75·0+	62·5
Dichloracetate	100 + + + 87·5+ +	75·0
Trichloracetate	112·5+ + + 100 + + 87·5+	75·0
Salicylate	100 + + + 87·5+ + 75 + +	62·5
Benzoate	125·0+ + + 112·5+	100·0

Hydrochlorides.

		0.
Sodium	100 + + + 87·5 + + 75·0 +	62·5
Ammonium	56·25+ + + 55·0 + + 43·75+	37·5
Monomethylamine	37·5 + + + 31·25+ + 28·12+ + 25 + + 21·87+	18·75
Trimethylamine	14·06+ + + 12·5 + + 10·93+ 9·73+	7·82
Dimethylamine	21·87+ + + 18·75+ + 15·62+ 14·06+	12·50
Tetraethylammonium	22·30+ + + 1·95+ + 1·75+ + 1·56+ 1·36+	0·97
Piperidine	6·25+ + + 5·46+ + 4·6 + + 3·9 + + 3·51+ 3·12+	2·69
Triethylamine	2·69+ + + 2·30+ + 1·95+	1·75
Benzylamine	3·51+ + + 3·12+ + 2·69+ + 2·20+	1·95
Isoamylamine	6·25+ + + 5·46+ + 4·60+ + 3·90+	3·51
Hexylamine	3·51+ + + 3·12+ + 2·69+ + 2·30+	1·95

Mastic.

One hundred cubic centimetres contained 0·13 per cent. solid, dried at 100° C.

Sodium Salts.

			0.
Formate	500 + + +	250 +	125
Lactate	500 +		250
Acetate	500 + +		250
Monochloracetate	500 + + +	250 + +	125
Dichloracetate	500 + + +	250 +	125
Trichloracetate	500 +		250
Salicylate	500		250
Benzoate	500		250
Benzene sulphonate	—		>500

(Hydro) Chlorides.

					0.
Sodium	112·5 + + +	100 + +	87·5 + +	75 +	62·5
Ammonium	75 + +	62·5 +			56·25
Monomethylamine	87·5 + + +	75 + +	62·5 +		56·25
Trimethylamine	62·5 + +	56·25 +			50·0
Dimethylamine	87·5 + + +	75 + +	62·5 +		56·25
Tetraethylammonium	—				>500
Piperidine	28·12 + + +	25 +			21·87
Triethylamine	28·12 + + +	25 + +	21·87 +	18·75 +	15·62
Benzylamine	14·06 + + +	12·5 + +	10·93 +		9·37
Isoamylamine	14·06 + + +	12·5 + +	10·93 +		9·37
Hexylamine	5·64 + + +	4·60 +			3·90

The following Table gives a summary of the chief results obtained.

The numbers indicate the salt solutions, the Arabic numerals representing the sodium salts, and the Roman numerals the hydrochlorides, the smaller numbers being given to those solutions with the higher surface tensions.

The numbers follow one another in the vertical columns in the order of their diminishing flocculating power.

The solutions with equal flocculating power are bracketed together.

Ferric hydroxide.	Zirconium hydroxide.	Cerium hydroxide.	Victoria blue.	Azo blue.	Brilliant Congo R.	Gold.	Arsenic sulphide.	Mastic.
8	⎧ 9	⎧ 9	8	XI	XI	XI	VI	XI
9	⎩ 8	⎩ 8	7	IX	IX	⎧ IX	IV	⎧ X
4	⎧ 7	3	10	X	I	⎩ VIII	IX	⎩ IX
3	⎪ 5	⎧ 5	⎧ 9	VII	⎧ X	X	⎧ XI	VIII
2	⎪ 4	⎨ 4	⎩ 6	⎧ III	⎪ VIII	⎧ VII	⎩ VII	VII
5	⎩ 2	⎩ 2	5	⎩ II	⎪ VII	⎨ VI	X	⎧ V
10	6	10	1	⎧ V	⎨ VI	⎩ IV	IV	⎨ IV
7	10	6	2	⎩ I	⎪ V	II	V	⎩ III
6	3	7	3	IV	⎪ IV	V	III	II
1	1	1	4	VIII	⎪ III	III	II	I
				VI	⎩ I	I	I	VI

Summary.

The theories regarding the flocculation of colloids by salts have been discussed. To test the "adsorption hypothesis," the precipitating capacity of salts containing organic ions, the normal solutions of which exhibit wide variations in their surface tensions, was determined. It was expected, should this hypothesis hold, that the salts giving solutions of lowest surface tension would exhibit the greatest flocculating capacity. The following sodium salts were employed: formate, acetate, lactate, mono-, di- and trichloracetate, salicylate, benzoate, and benzene sulphonate. Also the following (hydro) chlorides: sodium, ammonium, monomethylamine, trimethylamine, dimethylamine, tetraethylammonium, piperidine, triethylamine, benzylamine, isoamylamine, hexylamine. The following sols were used: ferric, zirconium and ceric hydroxides, Victoria blue B, azo-blue, brilliant Congo-red R, scarlet gold sol, arsenic sulphide, and mastic.

In general, no relationship was found to exist between the surface tensions of the normal solutions and the flocculating capacity of salts. When a series of salts was compared in which the varying ion was not the active precipitating ion, the precipitating limits were all within a comparatively narrow range, in spite of the wide variations of the surface tensions of the solutions. In the series, when the varying ion was the active ion, the range was large, although the precipitating capacity could not be generally correlated with the surface tension. The marked exception to this statement was shown in the case of mastic, when precipitated by the (hydro)chlorides. In this case the salts of low surface tension exhibit the greater flocculating power (with the exception of tetraethylammonium chloride).

It is suggested that suspensoid colloids may be subdivided into classes: (*a*) those, like ferric hydroxide, which owe their charge to association with "active" ions derived from salts from which they have been prepared, which are removed during aggregation, which results finally in flocculation, and which may be termed "exionic colloids;" (*b*) those owing their charge to the dissociation of the colloid itself, in which a rapidly diffusing ion (such as hydrogen ion) forms the outer layer, and is held electrostatically to a less diffusible ion. These may be termed "endionic colloids." Mastic is probably a colloid which belongs to this class.

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PROCEEDINGS
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THE ROYAL SOCIETY.

Series B. Vol. 90. **No. B 632.**

BIOLOGICAL SCIENCES.

CONTENTS.

	Page
Observations on Changes in the Blood Pressure and Blood Volume following Operations in Man. (Preliminary Communication.) By Captain H. C. BAZETT, M.C., R.A.M.C.	415
On the Cooling and Evaporative Powers of the Atmosphere, as Determined by the Kata-thermometer. By LEONARD HILL, F.R.S., and D. HARGOOD-ASH	438
The Influence of External Concentration on the Position of the Equilibrium attained in the Intake of Salts by Plant Cells. By WALTER STILES, M.A., Lecturer in Botany in the University of Leeds, and FRANKLIN KIDD, M.A., D.Sc., Fellow of St. John's College, Cambridge	448

PRINTED AND PUBLISHED FOR THE ROYAL SOCIETY BY
HARRISON & SONS, 44—47, ST. MARTIN'S LANE, LONDON, W.C. 2.
Price Three Shillings and Sixpence.

No. B 632.

April 1, 1919.

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Authors are requested to refer to a Memorandum on Mathematical Notation published in these 'Proceedings,' 1909, Series A, vol. 82, p. 14, and to adhere to the suggestions therein contained, so far as possible.

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Observations on Changes in the Blood Pressure and Blood Volume following Operations in Man. (Preliminary Communication.)

By Captain H. C. Bazett, M.C., R.A.M.C.

(Communicated by Prof. W. M. Bayliss, F.R.S. Received October 8, 1918.)

The cases here investigated were wounded men undergoing operations, and repeated examinations were usually made. Most of the cases showed only slight symptoms of shock.

Methods.—The systolic and diastolic blood pressures were measured before, during, and after operations, a Riva Rocci apparatus being used. The auscultatory method recommended by Oliver was used to determine the two levels. The hæmoglobin was estimated also, as far as possible, at the same time. The actual level of the hæmoglobin value was read by Haldane's method, while the changes in any patient were determined by comparison of the different samples in a Du Borscq colourimeter. For this purpose suspensions of the corpuscles in a dilution of 1 in 200 in saline were used, the volume chosen being 10 c.c., and these samples were hæmolysed with saponin before being read in the colourimeter. For this method I am indebted to Prof. Dreyer, and it has proved more accurate than any other. The blood has been taken always from either the ear or the finger. In estimating the blood volume changes from these readings, it has been assumed that the blood volume varies inversely as the hæmoglobin percentage. During and after operations this will be only relatively true, since hæmorrhage occurs. The amount of blood lost may, however, be roughly estimated by the loss of hæmoglobin in the first 24 hours after operation. In cases of slight shock, equilibrium will probably have been reached in this time. That this is true is indicated by the results obtained and put forward in Case I. In this patient a fair amount of blood was lost during the process of decompression for a fractured skull, and nearly all the blood lost was washed into buckets by a stream of saline running over the wound. The saline in these buckets was collected after the operation and the hæmoglobin content was determined by reading the contents in the Du Borscq colourimeter against a sample of the patient's own blood, taken before operation. In this way it was calculated that he lost 782 c.c. of blood. By the determination of the change in the hæmoglobin value in 24 hours, it was estimated that he lost 17·7 per cent. of his blood volume, and this was reckoned (taking Dreyer's formula for blood volume) to correspond to a loss of 760 c.c. The agreement was therefore remarkable, and it is probable that the methods are moderately accurate. In all the Tables the calculations of blood volume are made neglecting this factor of hæmorrhage. At the bottom of the Tables the estimated blood lost is given, and in the last column of the Tables corrected values for the blood volume are given in which the hæmorrhage has been approximately allowed for. The results obtained seemed to indicate that the changes in the hæmoglobin percentage of capillary blood do demonstrate the changes seen in the blood volume, provided that the lag due to a slow circulation and partial stasis is allowed for, the hæmoglobin changes following those in the blood pressure.

The results obtained are, briefly, that during the early stages of an operation the pulse rate, systolic, and pulse pressures are all raised, while the diastolic pressure is also usually slightly raised, and at this time the hæmoglobin percentage is slightly reduced, that is to say, the blood volume is probably increased. In the later stages of an operation, or in the post-operative stage, the blood-pressures all fall; the pulse rate may remain fairly fast, and with the fall of blood pressure there is a blood concentration. Thus it is seen that in most cases the blood volume curve runs parallel with the blood pressure, except that it usually lags about half an hour behind the other curve, and when the circulation is sluggish, as in cases of shock, it may lag an hour or more (compare Cases II and III). None the less, the two curves usually show a marked similarity. The cases investigated, as a rule, have shown very slight blood concentration, since nitrous oxide and oxygen anæsthesia was used in all cases in which shock was feared, and with this form of anæsthesia little or no shock was experienced. The pulse in even desperate patients was often actually improved by the amputation of a leg. Any ill effects following operation in these cases with nitrous oxide seemed to be attributable to either loss of blood in the operation or exposure to cold.

In one case (Case III) which was resplinted with no loss of blood, but considerable exposure of both lower limbs, there was a considerable fall of temperature, and this was the only case that showed real blood concentration to any marked degree. This case was anæsthetised with pure chloroform.

Since the curves always showed such a marked similarity, the diurnal variations were investigated to determine if they showed a similar relationship. One chart of these (Case IV) is included, and it will be seen that the relationship still holds good except for a brief half-hour after meals. These diurnal variations cannot be discussed now; I am indebted to Prof. Dreyer, who first drew my attention to these marked diurnal variations in the hæmoglobin percentage, for permission to mention this. He first worked out these diurnal variations in the hæmoglobin percentage, and I only mention them now in order that they may be compared with these post-operative changes.

Since, then, there appears to be this interdependence of the changes in the blood pressure and blood volume, it seemed necessary to explain it. An artificial schema was used in which a raised reservoir of water supplied the systolic pressure; a tap connected this with the artificial arterial system, this tap (representing the contraction of the heart) being opened at the rate of 30 to 40 times a minute by hand, the time being judged by a pendulum. The arterial system consisted of thin-walled rubber tubing, ending in a resistance created by capillary glass tubes, which could be changed. The water flowing through was collected and measured. It was found that the output per minute (MV) was proportional to the product of the pulse rate and pulse pressure.

$$\mathrm{MV} \propto \mathrm{PR} \times \mathrm{PP}.$$

It was also found that the resistance (R) of the artificial schema arteriole was indicated by the following formula:—

$$\mathrm{R} \propto \frac{\mathrm{MP}}{(\mathrm{PR} \times \mathrm{PP})^2},$$

where MP is the mean pressure and a high figure for R indicates a high resistance—but the proportion is not an arithmetical one. By the use of this formula it was easy to recognise, by the examination of the record, which of a series of capillary tube resistances had been used. Fig. A gives some examples of records obtained. In applying this formula, however, to blood pressure changes it did not appear to give reasonable results in cases where there were big changes of blood pressure.

However, on examination of the average pulse pressures found in man with different systolic pressures, as quoted for instance in Oliver's 'Studies in Blood Pressure,' it is found that the pulse pressure is a function of the square of the systolic pressure, if the blood circulation is otherwise normal. Thus, taking the figures from Oliver's book, a child of 15 has a systolic pressure of 107, and a pulse pressure of 33, a ratio of $\mathrm{PP}/(\mathrm{SP})^2$ of 0·00288. On the other hand, an arterio-sclerotic with a blood pressure of 165 has a pulse pressure usually of 75, a ratio $\mathrm{PP}/(\mathrm{SP})^2$ of 0·00275. The ratio is therefore remarkably constant. These results are charted in fig. B.

In considering the actual circulation, the viscosity of the blood must also be allowed for. Fig. C shows the probable changes in the viscosity of the blood for man with different percentages of hæmoglobin, this curve being

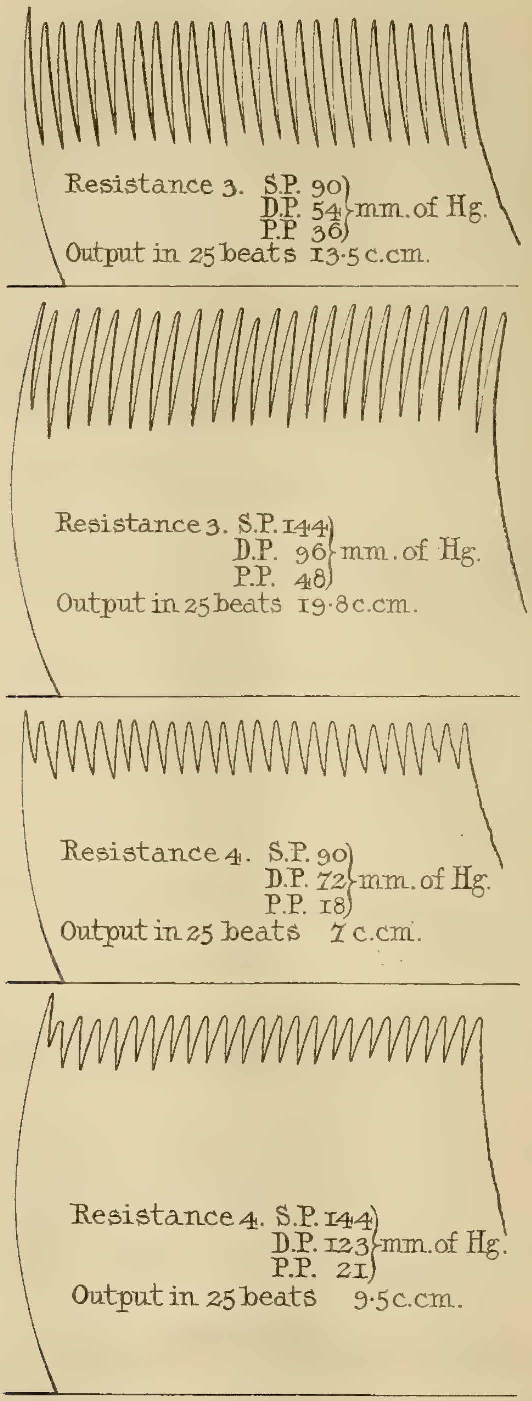
Resistance 3. S.P. 90
D.P. 54 mm. of Hg.
P.P 36
Output in 25 beats 13·5 c.cm.
Resistance 3. S.P. 144
D.P. 96 mm. of Hg.
P.P. 48
Output in 25 beats 19·8 c.cm.
Resistance 4. S.P. 90
D.P. 72 mm. of Hg.
P.P. 18
Output in 25 beats 7 c.cm.
Resistance 4. S.P. 144
D.P. 123 mm. of Hg.
P.P. 21
Output in 25 beats 9·5 c.cm.

Fig. A.

calculated from Arrhenius' formula for the viscosity of suspensions, and some actual figures for blood kindly supplied me by Prof. Bayliss.*

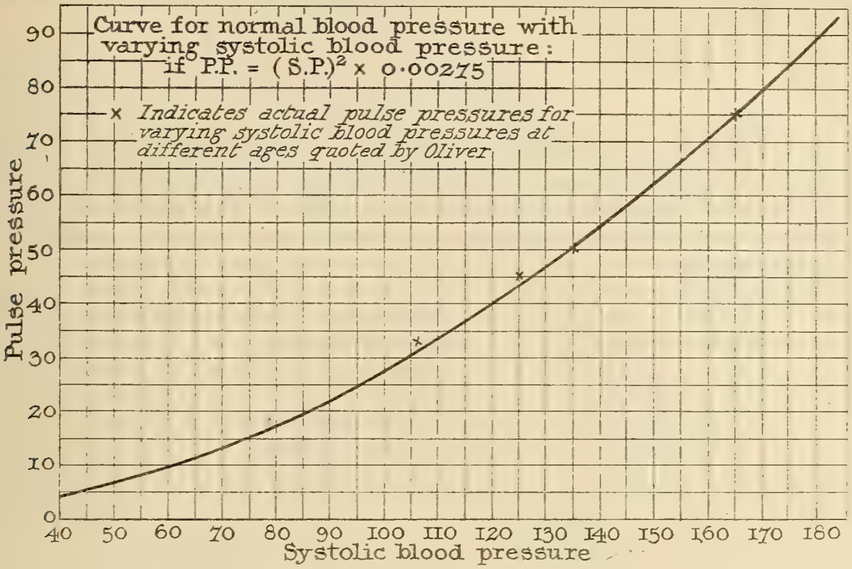

FIG. B.

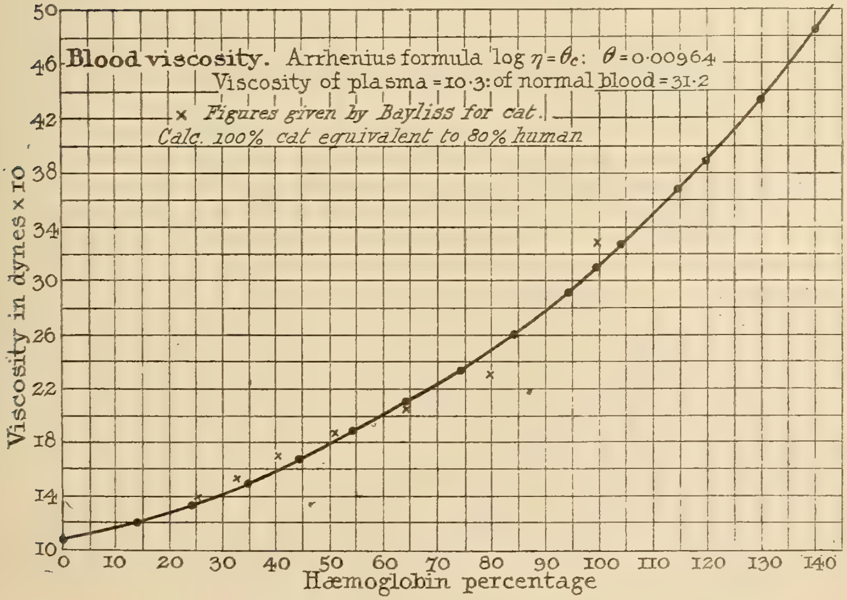

FIG. C.

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* Since this was written Trevan has published figures on viscosity changes ('Biochemical Journal,' vol. 12, p. 60), and has employed a different formula. His curve lies at a higher level, but is very similar to that given here within the limits of the hæmoglobin values seen in these patients.

So that the output of the heart per minute (MV) may be taken as being proportional to $PP/(SP)^2 \times PR$ with a normal value of 0·225, while the average resistance of the arterioles is represented by the formula

$$A \propto \frac{MP}{\eta \times [PP/(SP)^2 \times PR]^2},$$

where A is the resistance in the arterioles (a high figure indicating an increased contraction), MP is the mean pressure, η is the viscosity of the blood in dynes/1000, and the other figures are as before. The normal figure for A is about 60.

As modified in this way the formula gives results which usually corresponds with the blood volume changes actually observed, an increased vasomotor tone as estimated by the blood pressure changes being followed by a blood concentration. The interrelationship of the blood pressure and hæmoglobin percentage curves can then be explained. It will also be seen that in the case of diurnal variations, or patients with only slight shock (see Cases II, III, and IV), the value of A may give a graph nearly parallel with the diastolic pressure changes. On the other hand, the relationship between the value of A and blood volume changes is not obvious in certain cases, especially in cases with nitrous oxide and oxygen anæsthesia, and in cases where the blood pressure falls to a very low level, where the changes cover a big range. Probably changes in venous tone are also of great importance, since venous contraction is often induced in septic patients under nitrous oxide and oxygen anæsthesia.* It is possible, too, that the curve for the value of pulse pressures does not hold good for very low pressures. With high blood pressures the elasticity of the arteries may depend largely on the fibrous and muscular coats, while with low pressures, the elasticity may resemble much more that of an elastic rubber tube. So that, while the formula seems to hold well for pressures above 100 mm., it is very possible that the pulse pressures should be divided by $(100)^2$ and not by the (systolic pressure)^2, whenever the systolic pressure is below 100 mm. of mercury. Also, with very rapid pulse rates, it may prove necessary to take into consideration the shortening of the duration of systole.

None the less the formula seems to hold good for most conditions. It demonstrates the fact that under circumstances such as exercise and excitement, the rise of systolic pressure is accompanied by an actual lowering of the average vaso-motor tone, the increased heart output more than compensating for the vaso-dilatation, and so raising the systolic pressure. This agrees with the fact that during exercise the diastolic pressure may fall.

---

* It would seem probable that changes in the blood volume may be associated with alterations in the capacity of the arterial system, when parallel changes will be seen in the blood pressure, or with alterations in the capacity of the veins, when the blood pressure is only indirectly affected.

In the curves of diurnal variations the lag of the hæmoglobin changes in the capillary blood behind the blood pressure changes is very evident, and the hæmoglobin change is therefore probably secondary. On the other hand, the concentration seen in the capillary blood immediately after a meal occurs quickly, is transient, and has no parallel in the blood pressure changes; it is probably a local and temporary change due to a local constriction of the blood vessels. By reference to the Tables of Case IV it will be seen that an estimation of the hæmoglobin of venous blood immediately after a meal showed it to be almost 10 per cent. more dilute than the capillary blood, while the capillary blood itself showed this dilution a little later. So that all the figures seem explicable, if it be assumed that any change in the hæmoglobin percentage of the capillary blood, *unless it be a transient one,* indicates a corresponding change in the blood volume. In cases where the peripheral circulation is reduced to a low level (as in Case III), a considerable degree of stasis may result, and then the change in the capillary blood may lag far behind the general circulation changes.

One case of spinal anæsthesia (Case X) is included, and though an examination of the blood changes was not possible in this case, yet the changes observed are easily explicable when analysed by the formula—the stovaine inducing a fall of blood pressure through a partial vasomotor paralysis, and this being compensated for by an increased heart output until the resistance is raised by vaso-constriction in other parts. One case (Case XII) of pure traumatic shock uncomplicated by hæmorrhage or anæsthesia is also included for comparison. In this case collapse occurred with great dilution of the blood, and this is paralleled by a calculated great loss of vasomotor tone.

This formula is therefore put forward tentatively as of value in the analysis of the circulatory changes in most clinical conditions. It is not claimed that its truth is absolute, and it can probably be improved.

In conclusion, my thanks are due to Prof. Dreyer and Prof. Bayliss for much valuable advice, to Lt.-Col. Waring, D.S.O., for the facilities he gave me to carry on this research, and to Captain Wagstaffe, F.R.C.S., for assistance in many of the cases, and for access to all his patients.

Case I.—(Not Charted.) Table of Observations and Calculations.

Gnr. F——. Penetrating wound of head. Wound evening of 23.9.17. Anæsthetic, C.E. and $CHCl_3$. Trephined. Recovery.

Date.	Time.	Remarks.	P.R.	S.P.	D.P.	P.P.	M.P.	Hb.	Visc.	M.V.	A.	Bl. Vol.	B.V. (corr.).
24.9.17	6.15 P.M.	On admission	74	135	85	50	110	92	28·2	0·203	94·5	102	102
25.9.17	2.50 P.M.	—	86	122	80	42	101	94	29	0·242	59·3	100	102
	3.5	Anæsthetised											
	3.15	Shaving	72	122	85	37	103	—	—	0·179	111		
	3.28	Shaving	—	—	—	—	—	94·5	29·2	—	—	99·5	99·5
	3.30	Operation started											
	4.7	Operation finished	—	—	—	—	—	91	28	—	—	103	85
	4.20	Rectal saline ⊙i											
	4.55	In ward	120	106	70	36	86	87·7	27	0·385	26·5	107·3	92
	7.0	—	120	104	77	27	90	86·5	26·5	0·300	43·7	115·5	91
26.9.17	3 P.M.	—	100	127	86	41	106	77·3	24·3	0·255	67·1	100	100

Blood lost collected in buckets, with saline used, estimated as 782 c.c. Blood lost = 17·7 per cent. of blood volume = 760 c.c.

Case II.—Table of Observations and Calculations.

Pte. H——. Excision of knee joint. C.E. Open ether. (Shipway.) Recovery.

Date.	Time.	Remarks.	P.R.	S.P.	D.P.	P.P.	M.P.	Hb.	Visc.	M.V.	A.	Bl. Vol.	B.V. (corr.).
21.6.17	1.0 P.M.	Excited	116	145	85	60	115	64	21	0·331	57·5	—	100
	1.5	Quieter	116	138	90	48	112	64	21	0·293	72·5	—	100
		Scopolamine Morphine Atropine injection.											
	2.15	Theatre	104	140	85	55	113·5	63·5	20·9	0·292	71·8	100	100
	2.27	Anæsthetised	120	152	95	57	123·5	—	—	0·296	77		
	2.35	Operating	128	155	92	63	123·5	—	—	0·331	63·6		
	2.37	Sawing bone	128	155	90	65	122·5	61·8	20·3	0·346	56·8	101·7	98
	2.44	Stitching	132	142	78	64	110	57·2	19·5	0·418	36	110·6	100
	2.50	Dressing wound	140	145	92	53	118·5	—	—	0·353	54·4		
	3.0	Leaving theatre	136	140	85	55	112·5	61·4	20·3	0·378	44	103·2	93
	4.0	Ward	108	128	80	48	104	60·6	20	0·315	58·9	104·7	95
	5.45	Ward	100	128	80	48	104	65·3	21·2	0·292	65·9	97	87
	7.20	Ward	100	128	85	43	106·5	67·8	22	0·262	81·2	93	83
22.6.17	12.45 P.M.	—	112	135	90	45	112·5	56	19·1	0·276	86	100	100

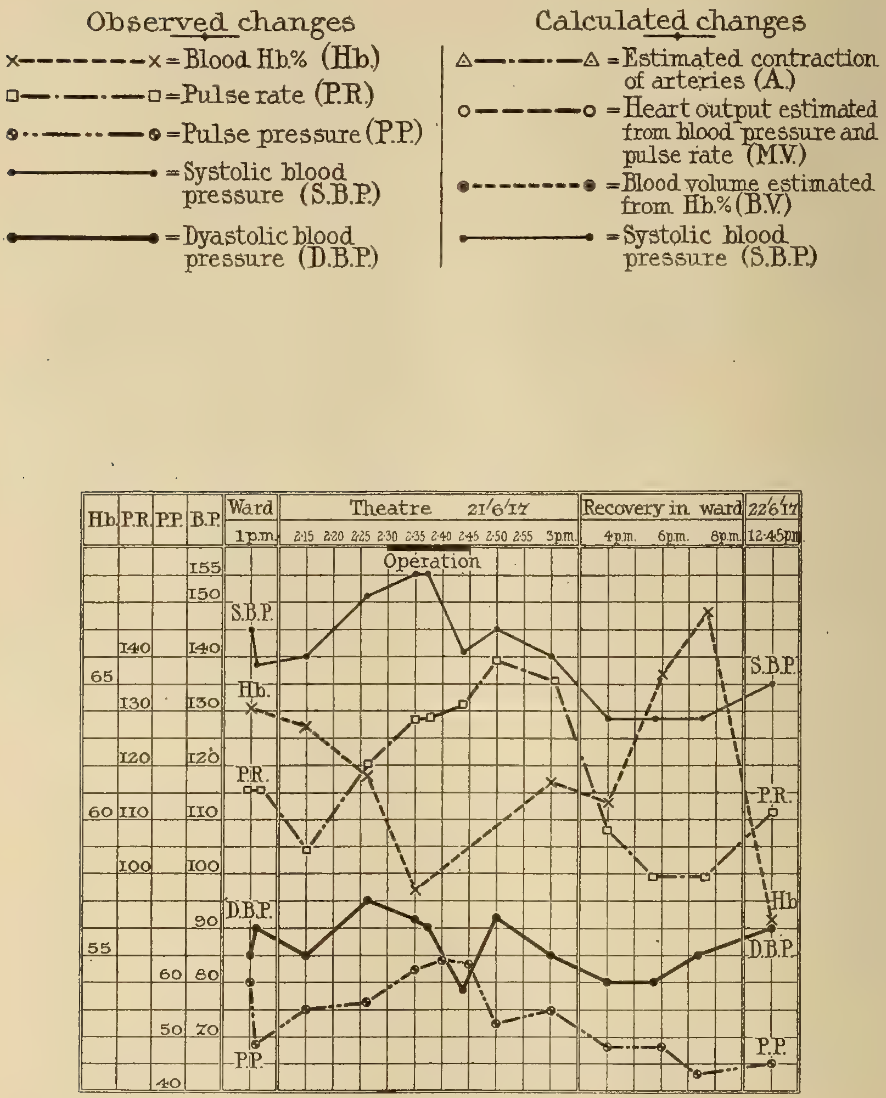
Blood lost = 12·5 per cent. of blood volume = about 480 c.c.

Case III.—Table of Observations and Calculations. (See Charts.) Effects due probably chiefly to cold.

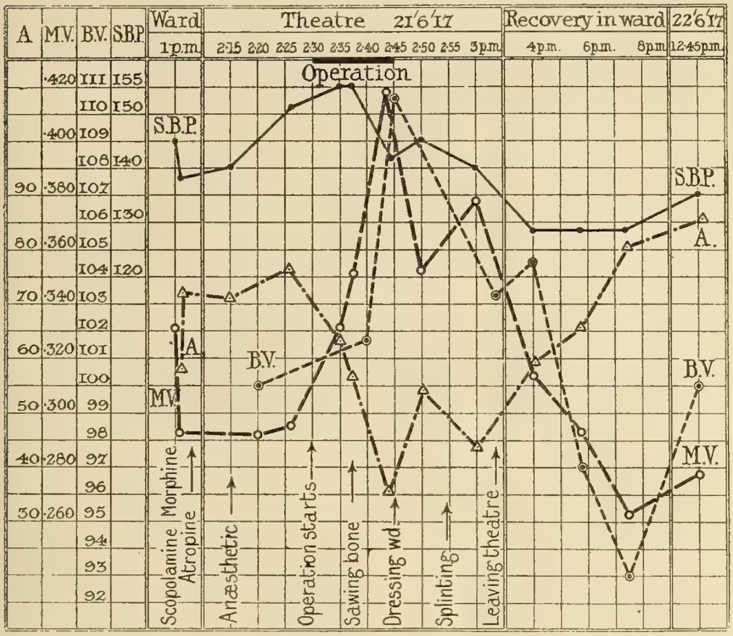
Pte. B——. $CHCl_3$. Resplinting. Exposure to cold. Recovery.

Date.	Time.	Remarks.	P.R.	S.P.	D.P.	P.P.	M.P.	Hb.	Visc.	M.V.	A.	Bl. Vol.	B.V. (corr.).
23.10.17	12.0	Ward	96	133	88	45	110	82	25·1	0·244	73·7	100	100
24.10.	1.10 P.M.	Ward (excited)	96	130	70	60	100	84	26·1	0·341	32·9	100	100
	1.55	Theatre	96	126	72	54	99	83·2	26	0·336	33·7	100·7	100·7
	2.25	Anæsthetised	80	112	76	36	94	84	26·1	0·229	63·7	99·9	99·9
	3.50	Back in ward	60	110	76	34	93	86·5	26·5	0·169	128·0	95·8	95·8
	5.25	Shivering	80	118	76	42	97	101·7	31·5	0·241	53·0	82·3	82·3
	7.20	Subnormal temperature	90	126	76	50	101	99·3	31	0·284	40·3	84·3	84·3
25.10.	11.40 A.M.	Comfortable	90	128	82	46	105	84	26·1	0·253	62·8	100	100

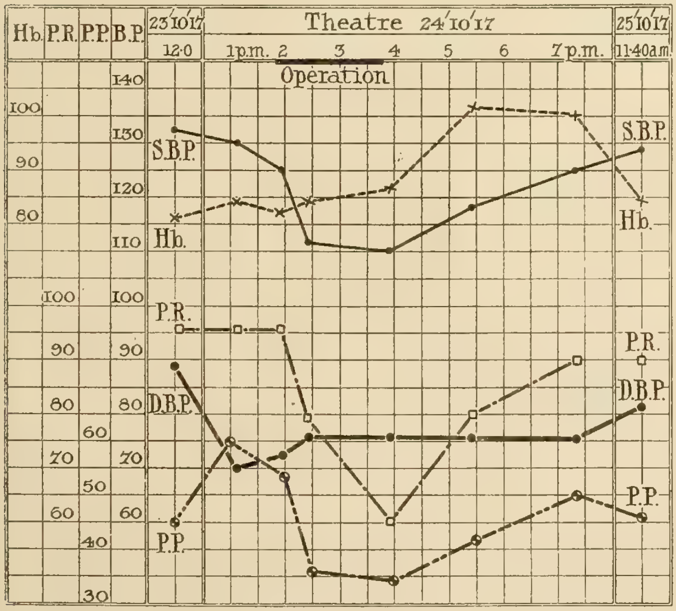
Blood lost—nil.


Observed changes
×——×=Blood Hb.% (Hb.)
□——□=Pulse rate (P.R.)
●——●=Pulse pressure (P.P.)
●——●=Systolic blood pressure (S.B.P.)
●——●=Dyastolic blood pressure (D.B.P.)
Calculated changes
△——△=Estimated contraction of arteries (A.)
○——○=Heart output estimated from blood pressure and pulse rate (M.V.)
●——●=Blood volume estimated from Hb.% (B.V.)
●——●=Systolic blood pressure (S.B.P.)
Hb.
P.R.
P.P.
B.P.
Ward
1p.m.
Theatre 21/6/17
2·15 2·20 2·25 2·30 2·35 2·40 2·45 2·50 2·55 3p.m.
Recovery in ward
4p.m. 6p.m. 8p.m.
22/6/17
12·45pm
Operation
155
150
140
140
65
130
130
120
120
60
110
110
100
100
90
55
60
80
50
70
40
S.B.P.
Hb.
P.R.
D.B.P.
P.P.
S.B.P.
P.R.
Hb
D.B.P.
P.P.

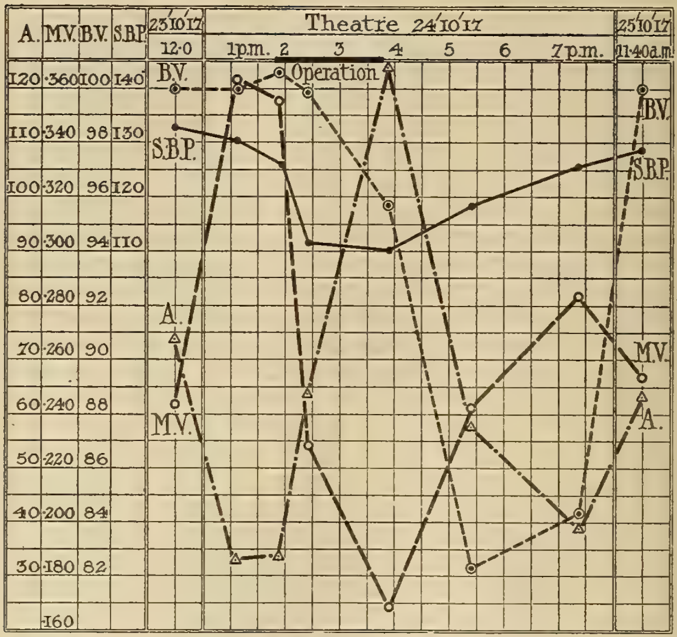
Case II.



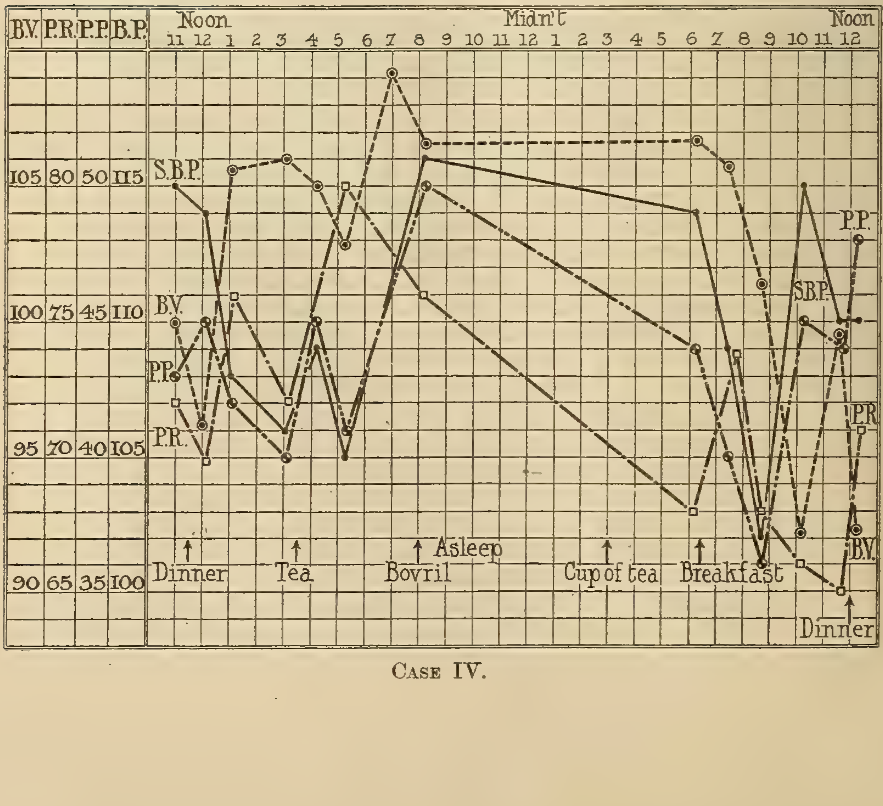
CASE II (*continued*).



CASE III.



CASE III (*continued*).

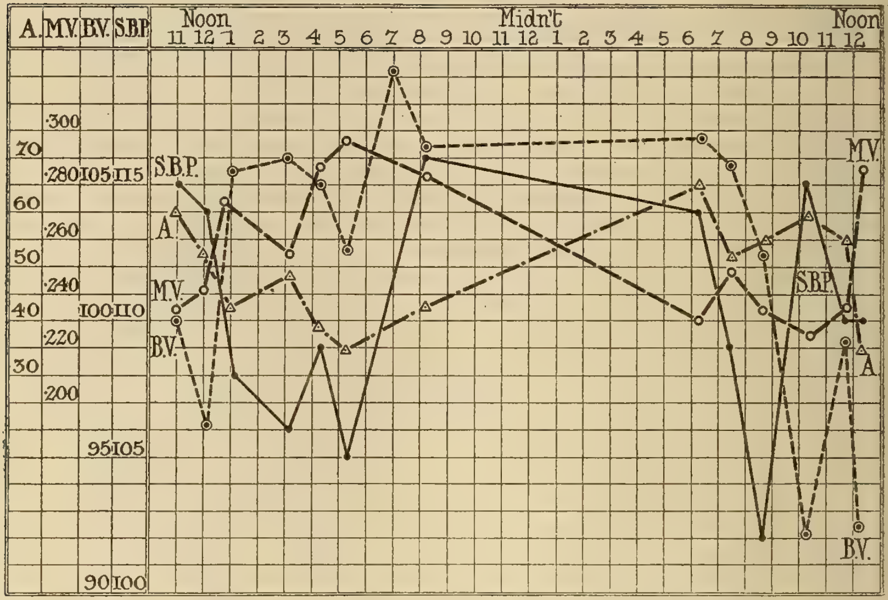


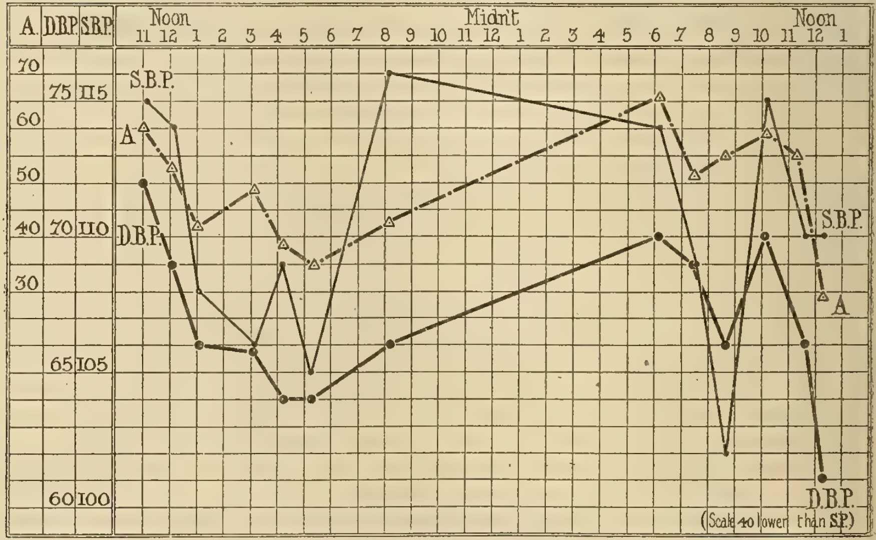
CASE IV.

Case IV.—Table of Observations and Calculations.

Pte S——. G.S.W. Abdomen. Apyrexial. Diurnal Variations at Rest in Bed. Meals early.
"Summer" time 20.7.17–21.7.17.

Date.	Real time.	Remarks.	P.R.	S.P.	D P.	P.P.	M.P.	Hb.	Visc.	M.V.	A.	Bl. Vol.
20.7.17	11.5 A.M.		72	115	72	43	93·5	92·5	28·5	0·234	59·8	100
	11.30	*Dinner*										
	12.5 P.M.	—	70	114	69	45	91·5	96·2	29·6	0·242	52·8	96·1
	1.5	—	76	108	66	42	87	87·8	27·5	0·273	42·4	105·6
	3.5	—	72	106	66	40	86	87·2	27·1	0·256	48·4	106
	3.30	*Tea, etc.*										
	4.15	—	76	109	64	45	86·5	88	27·5	0·287	38·2	105
	5.20	—	80	105	64	41	84·5	90	27·8	0·297	34·4	102·7
	7.0	—	—	—	—	—	—	84·7	26·1	—	—	109·2
	8.15	*Bovril*	76	116	66	50	91	86·8	26·5	0·283	42·8	106·5
		Asleep										
21.7.17	3.0 A.M.	*Tea*										
	6.15	—	68	114	70	44	92	86·6	26·5	0·230	65·5	106·7
	6.20	*Breakfast*										
	7.30	—	74	109	69	40	89	88·5	27·6	0·249	52·0	105·8
	8.40	—	68	102	66	36	84	90·2	27·8	0·235	54·7	102·4
	10.15	—	66	115	70	45	92·5	100·5	31·4	0·224	48·7	92·1
	11.40	—	65	110	66	44	88	93	28·7	0·236	55	99·3
	11.45	*Dinner*										
	12.10 P.M.	*Blood from vein*	—	—	—	—	—	91·5	28·3			
	12.15	—	72	110	62	48	86	100·2	31·3	0·286	33·6	92·3





CASE IV (*continued*).

Case V.—Table of Observations and Calculations.

Pte. O——. G.S.W. Compound fracture neck of femur. Very septic. Vomiting, etc. Wound enlarged on fourth day. Gas infection probably. Died five days later of septicæmia c̄ jaundice.

Date.	Time.	Remarks.	P.R.	S.P.	D.P.	P.P.	M.P.	Hb.	Visc.	M.V.	A.	Bl. Vol.
	11.20 A.M.	Ward	128	102	75	27	88·5	70	22·5	0·332	25·7	100
	12.15 P.M.	$CHCl_3$										
	12.20	—	—	—	—	—	—	61·5	20·3	—	—	113·8
	12.35	Operation finished. Fixing splint	—	—	—	—	—	61	20·2	—	—	114·5
	12.40	Operation finished completely	—	—	—	—	—	63	20·5	—	—	111
	12.45	—	160	105	85	20	95	—	—	0·29	55·0	
	12.50	Alkaline saline infusion (hypertonic) ⊙i in 10 min. intravenously.										
	1.0 P.M.	—	—	110	85	25	97·5	—	—	? 0·32	49	
	1.5	Leaving theatre	—	—	—	—	—	57·5	19·5	—	—	121·8
	2.0	In ward	180	85	66	19	75·5	—	—	0·475	16·4	
	2.20	Rectal saline pituitrin	168	—	—	—	—	62	20·4	—	—	112·5
	3.30	Intravenous saline ⊙i										
	4.30	Sweating										
	6.15	—	132	—	—	—	—	62·5	20·4	—	—	111·5

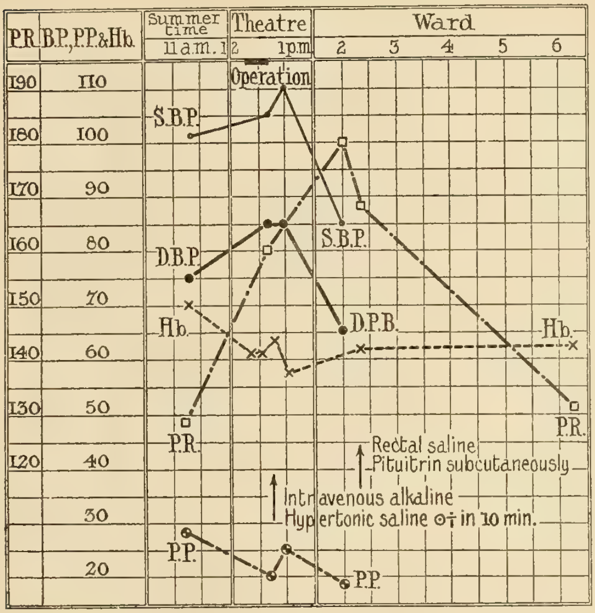
Blood lost very little, but not determined.

Case VI.—Effects probably due chiefly Hæmorrhage. Table of Observations (see Chart) and Calculations.

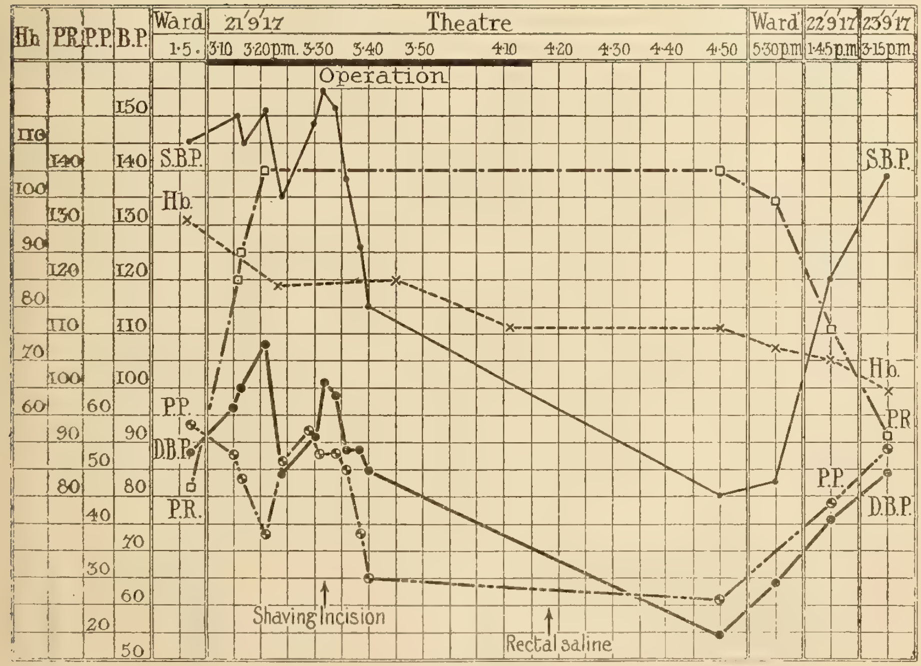
Pte. S——. G.S.W. Head. Penetrating. Hæmorrhage from middle meningeal artery and other wounds. C.E. and pure $CHCl_3$. Trephined, 21.9.17. Recovery.

Date.	Time.	Remarks.	P.R.	S.P.	D P.	P.P.	M.P.	Hb.	Visc.	M.V.	A.	Bl. Vol.	B.V. (corr.).
20.9.17	1.25 P.M.	On admission	58	140	85	55	112	100	31·2	0·163	135	100	96·5
	2.45	Ward. Restless	58	142	85	57	113	—	—	0·164	135		
	7.15	Ward	52	146	78	68	112	100	31·2	0·166	130	100	96·5
21.9.17	1.5 P.M.	Ward	82	146	88	58	117	96·5	29·7	0·224	78·5	100	100
	3.16	Anæsthetic, 3.10	120	150	97	53	123	—	—	0·283	51·7		
	3.17	—	130	145	99	48	123	—	—	0·297	48		
	3.21	Shaving	140	146	108	38	127	—	—	0·249	69		
	3.24	—	125	135	84	51	110	—	—	0·350	30·3		
	3.30	—	128	148	92	56	120	84	26·0	0·327	43·2	115	115
	3.32	Incision	124	155	102	53	128	—	—	0·274	65·6		
	3·34	Incision	115	152	99	53	125	—	—	0·264	68·9		
	3.36	—	140	138	88	50	113	—	—	0·367	32·3		
	3.38	—	—	126	88	38	107						
	3.40	Clipping bone	—	115	85	30	100						
	3.45	First flap replaced	—	—	—	—	—	85	26·2	—	—	113·5	103
	4.12	Stitching second wound	—	—	—	—	—	75·5	23·6	—	—	128	95
	4.50	Rectal saline, 4.18	140	80	54	26	67	75·6	23·6	0·570	8·75	127·5	94·5
	5.30	—	134	82	64	18	73	72·5	—	0·358	24·2	133	97·5
22.9.17	1.45 P.M.	—	112	120	76	44	98	70	22·7	0·342	36·9	? 100	? 100
23.9.17	3.15	Sl. sepsis	92	138	84	54	111	64	21·0	0·261	77·5	? 100	? 100

N.B.—Considerable loss of blood with rapid replacement of fluid lost. Blood lost = 27·5 per cent. of blood volume or more = 1230 c.c.



CASE V.



CASE VI.

Case VII.—Table of Observed Changes and Calculations.

Sgt. D——. G.S.W. Multiple penetrating wound of knee. Amputation of leg through thigh. N_2O and O_2. Reactionary hæmorrhage. Death from sepsis a week later. Times in summer time.

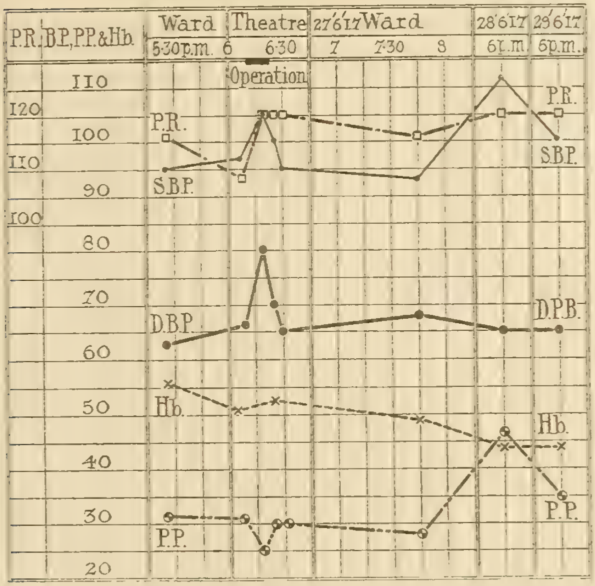
Date.	Time.	Remarks.	P.R.	S.P.	D.P.	P.P.	M.P.	Hb.	Visc.	M.V.	A.	Bl. Vol.	B.V. (corr.).
27.6.17	5.25 P.M.	Ward	116	95	63	32	79	56	19	0·412	24·6	100	100
	6.7	In theatre	108	97	66	31	81·5	—	—	0·356	25·7	—	—
	6.10	In theatre	—	—	—	—	—	51	18	—	—	109·9	109·9
	6.11	Anæsthetic											
	6.20	Operation ending	120	105	80	25	93	—	—	0·272	68	—	—
	6.25	Dressing wound	—	—	—	—	—	53	18·5	—	—	105·2	96
	6.27	—	120	100	70	30	85	—	—	0·360	35·5	—	—
	6.30	Leaving theatre	120	95	65	30	80	—	—	0·399	27·2	—	—
	7.45	Ward. Bleeding	116	93	68	27	81·5	49	17·8	0·362	35	114·9	? 90
28.6.17	6 P.M.	Better	120	112	65	47	88·5	44	16·8	0·45	26	100	100
29.6.17	6 P.M.	Not so well	120	100	65	35	82·5	44	16·8	0·42	27·9	100	100

Case VIII.—Table of Observed Changes and Calculations.

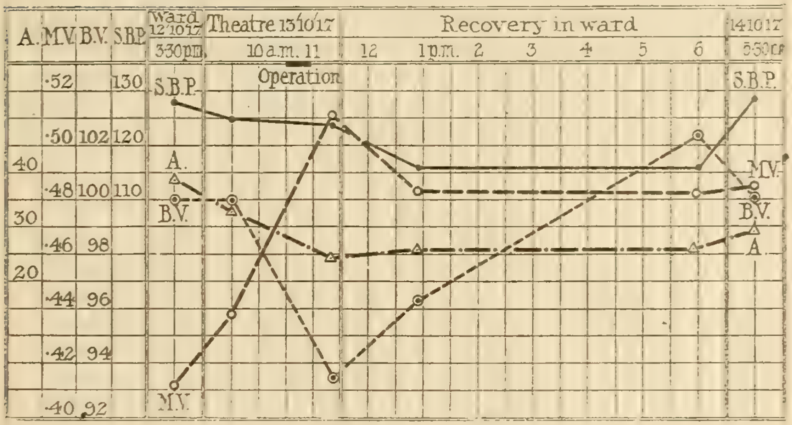
Pte. H——. Amputation through thigh. N_2O and O_2. Recovery.

Date.	Time.	Remarks.	P.R.	S.P.	D.P.	P.P.	M.P.	Hb.	Visc.	M.V.	A.	Bl. Vol.	B V. (corr.).
12.10.17	3.30 P.M.	Ward	116	128	70	58	99	36	15·2	0·412	38·5	100	100
13.10.17	9.30 A.M.	Ward	116	125	66	59	96	33	14·7	0·438	33·9	100	100
	10.30	Operation (25 mins.)											
	11.20	Ward	140	124	68	56	96	35·3	15·1	0·510	24·5	93·5	82
	12.55 P.M.	Ward	136	116	68	48	92	34	15·0	0·484	26·3	96·3	85
	6.0	Ward	130	116	66	50	91	32·5	14·6	0·483	26·7	101·3	91
14.10.17	5.30 P.M.	—	128	128	66	62	97	29	14·0	0·484	29·6	100	100

Some symptoms of shock. Extremely septic; operation slow and moderate hæmorrhage. Blood lost 12·1 per cent. of blood volume = about 545 c.c.



CASE VII.



CASE VIII.

Case IX.—Table of Observations and Calculations (not charted).

Pte. J——. G.S.W. Compound fracture of femur involving hip joint. N_2O and O_2. Incised drained. Recovery.

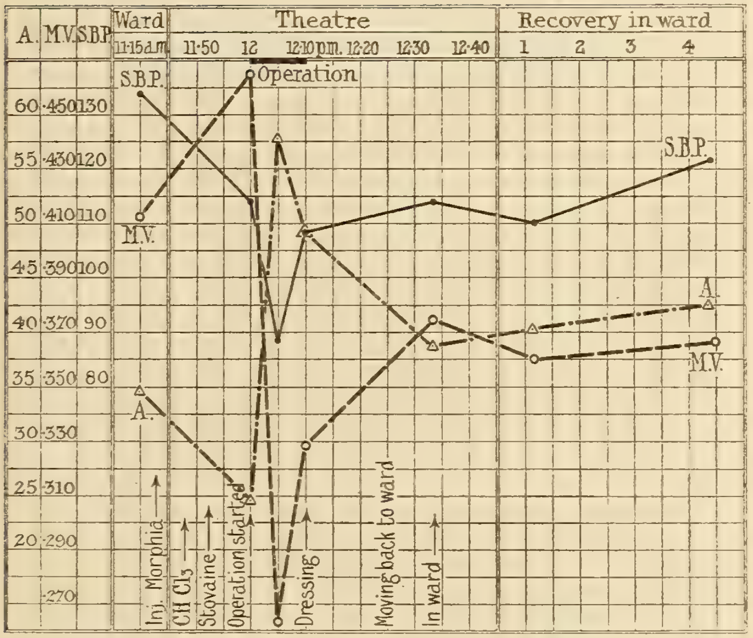
Date.	Time.	Remarks.	P.R.	S.P.	D.P.	P.P.	M.P.	Hb.	Visc.	M.V.	A.	Bl. Vol.	B.V. (corr.).
11.10.17	11.45 A.M.	Ward	128	138	100	38	119	80	25	0·256	72·6	100	100
	12.30 P.M.	Operation											
	1.30	Ward	112	138	94	44	116	83	25·5	0·259	67·8	96	88·5
	6.0	—	108	146	92	54	119	88	27·3	0·274	58·2	91	83·5
12.10.17	3.15 P.M.	—	92	130	86	44	108	74	23·5	0·239	80·5	100	100

Blood lost 7·5 per cent. of blood volume = about 350 c.c. Veins very engorged before operation; this disappeared after operation.

Case X.—Table of Observed Changes and Calculations.

Pte. F——. Amputation through thigh under spinal anæsthesia. Stovaine 0·6 grm. after a few minutes $CHCl_3$. Recovery. January, 1918.

Date.	Time.	Remarks.	P.R.	S.P.	D.P.	P.P.	M.P.	Hb.	Visc.	M.V.	A.	Bl. Vol.
1.0.18	11.15 A.M.	Ward	128	134	76	58	105	? 60	17·7 to 20	0·413	34·9	?
	11.52	Stovaine										
	12.0	Skin incision	138	114	70	44	92	?	?	0·467	24·9	?
	12.5 P.M.	—	116	88	54	34	71	?	?	0·264	57·7	?
	12.10	Dressing	128	108	78	30	93	—	—	0·329	48·2	?
	12.33	In ward	128	114	76	38	95	?	?	0·375	38·2	?
	1.10	In ward	128	110	76	34	93	?	?	0·360	40·6	?
	4.25	In ward	124	122	78	44	100	?	?	0·367	42	?


A.
M.V.
S.B.P.
Ward
Theatre
Recovery in ward
11·15 a.m.
11·50
12
12·10 p.m.
12·20
12·30
12·40
1
2
3
4
60 ·450 130
55 ·430 120
50 ·410 110
45 ·390 100
40 ·370 90
35 ·350 80
30 ·330
25 ·310
20 ·290
·270
S.B.P.
Operation
M.V.
S.B.P.
A.
A.
M.V.
Inj. Morphia
CH Cl$_3$
Stovaine
Operation started
Dressing
Moving back to ward
In ward

Case X.

Case XI.—Effect on Recipient of Blood Transfusion of about 600 c.c.

L.-Cpl. P——. Blood Transfusion.

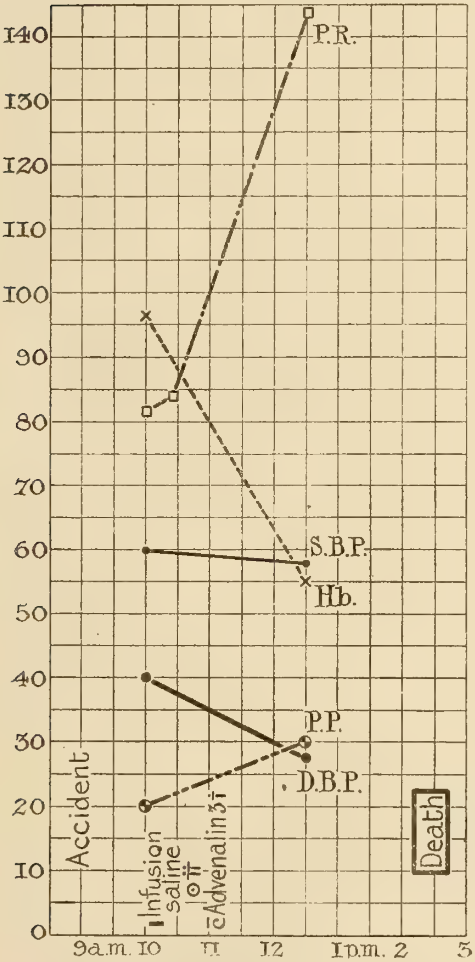
Date.	Time.	Remarks.	P.R.	S.P.	D.P.	P.P.	M.P.	Hb.	Visc.	M.V.	A.	Bl. Vol.
16.6.17	6.45 P.M.	Before transfusion	120	115	70	45	93	24	13·4	0·408	41·7	100
	7.55	After transfusion	108	128	58	60	98	33	14·5	0·396	43·2	115
17.6.17	6.15 P.M.	Very fit	104	128	75	53	102	38	15·5	0·337	57·8	100

Case XII.—Table of Observed Changes and Calculations.

Pte. W——. Railway accident. One leg torn off in thigh. Other foot and one arm crushed. No exposure to cold. No improvement with warmth.

Date.	Time.	Remarks.	P.R.	S.P.	D.P.	P.P.	M.P.	Hb.	Visc.	M.V.	A.	Bl. Vol.
	9.0 A.M.	Accident										
	10.0	In ward	89	60	40	20	50	97	30	0·444	8·4	? 100
	10.5	Intravenous saline ⊙ii c̄. ʒi adrenalin. Slight improvement only in pulse.										
	12.30 P.M.	—	144	58	28	20	43	55·5	19	1·29	1·4	175

Death at 2.30 P.M. Patient was extremely difficult to bleed from ear at 10 A.M., but bled easily at 12.30 P.M.


140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
9a.m.
10
11
12
1p.m.
2
3
P.R.
S.B.P.
Hb.
P.P.
D.B.P.
Accident
Infusion saline ʘ ii
c̄ Advenalin ʒi
Death

CASE XII.

On the Cooling and Evaporative Powers of the Atmosphere, as Determined by the Kata-thermometer.

By Leonard Hill, F.R.S., and D. Hargood-Ash.

(Department of Applied Physiology, Medical Research Committee.)

(Received December 3, 1918.)

Part I.—*Cooling Power in Moving Air. The Kata-thermometer as an Anemometer.*

In a paper published in 'Phil. Trans.' (B, vol. 207, 1916, pp. 183–220) by L. Hill, O. W. Griffiths, and M. Flack, there was detailed the theory and use of an instrument, the kata-thermometer, a large-bulbed alcohol thermometer, for determining the cooling power of the atmosphere on a surface at body temperature. A formula $H/\theta = 0{\cdot}27+0{\cdot}36\sqrt{V}$, where $H =$ heat lost in mille-calories per square centimetre per second, $\theta = (36{\cdot}5-t)^\circ$ C., where $t =$ temperature of enclosure, and $V =$ velocity of air current in metres per second, was obtained for the loss of heat of the dry kata-thermometer in a current of air; 36·5° C. was chosen as the skin temperature. This is a variable, and only reaches that figure in warm atmospheres.

The constant 0·36 in the above formula was determined from experiments which were carried out with the apparatus then available in a tube of which the cross-section area was of the same order of magnitude as that of the kata. Therefore, in calculating the velocity of the air current, *i.e.*, the mean velocity of the air striking the kata, the area of cross-section of the kata was subtracted from that of the tube.

In later experiments the authorities of the East London College have been good enough to allow us the use of the large wind tunnel and other smaller tunnels established for aeroplane observations. Mr. N. A. V. Piercy, the lecturer on Aeronautical Engineering, has kindly helped us to determine very exactly the velocity of the wind in the tunnels.

In all three tunnels used the air was drawn through the tunnel by means of a large electric fan, and the velocity deduced from the difference of pressure inside and outside the tunnel, the gauges used being calibrated by means of a Pitot tube. All the tunnels were of square cross-section. The largest tunnel used was 48 inches wide and made of steel. The pressure difference was read from tilted gauges, the velocity being given by the formula :—

$$V = K\sqrt{d},$$

where V is the velocity in metres per second, K a constant depending upon the gauge and tunnel and determined by comparison with a Pitot tube, and d the difference of level of the liquid in the two arms of the gauge.

In all the observations taken the kata was heated in hot water in a Thermos flask, being kept in the water till the air space at the top was about half full of alcohol. The same kata, stop-watch, and thermometer, the last graduated to 0·1° C., were used throughout the experiments. In the Royal Society paper cited above it is detailed how a factor is determined for each kata by which the readings can be expressed as cooling powers in mille-calories per square centimetre per second.

The value for the kata factor was re-determined before being used in an enclosure of still air jacketed with water, the results obtained being as follows:—

Mean time of cooling = 105·9 seconds.
Temperature of enclosure = 17·8° C.
$\theta = 36{\cdot}5^\circ - 17{\cdot}8^\circ = 18{\cdot}7^\circ$ C.

The factor $= 0{\cdot}27\theta \times$ mean time of cooling (105·9) = 535. The graph obtained from the result of these later experiments and plotted in a similar manner to that obtained from the earlier ones, gives the value of the constant a in the formula $H/\theta = 0{\cdot}27 + a\sqrt{V}$ as 0·49. The figures obtained are given in Table 2.

The difference in the value of the constant found from the two sets of experiments suggested that the true mean velocity had not been obtained in the earlier work, and on re-considering the problem it appeared probable that if half the area of cross-section of the kata in place of the whole were subtracted from the sectional area of the tube used in the earlier work a more correct value of the velocity would be given. Calculating the velocity in this way the value of the constant becomes $0{\cdot}36 \times 1{\cdot}32 = 0{\cdot}48$, which is in close agreement with the value obtained from the later experiments.

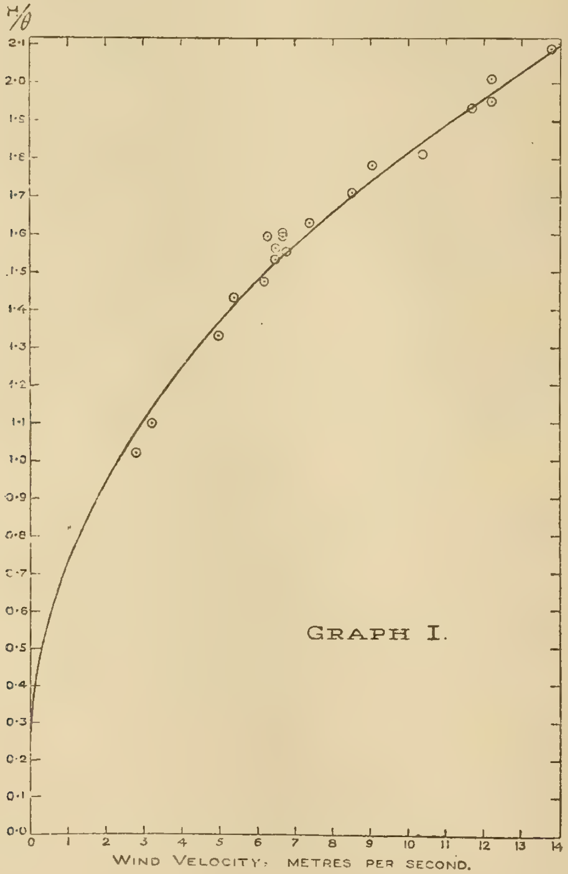
We tested the new constant by using it in measuring the velocity of the wind with the kata against the standard anemometers at Kew Observatory, and we are much indebted to Dr. Chree for the facilities he gave us to do this.

A succession of kata readings were taken during each period of time and the mean of these taken, while the anemometer records were continuous. An extra strong gust may thus be missed by the kata, if no reading happened to be taken at that moment. This possibly was the case in the third series of observations given in Table 1.

Table 1 shows the values obtained for wind velocities by a Robinson's cup anemometer, a Dines pressure tube, and a kata-thermometer. The observations were taken on the roof of Kew Observatory. The day was one with a steady strong breeze :—

Table 1.

Time.	Wind velocities (metres per sec.).		
G.M.T.	Cup anemometer.	Pressure tube.	Kata.
x 40 – x 50	6·0	6·5	6·4
x 55 – xi 5	6·5	6·25	6·6
xi 10 – xi 20	6·0	5·1	5·2
xi 20 – xi 30	6·4	5·7	6·3



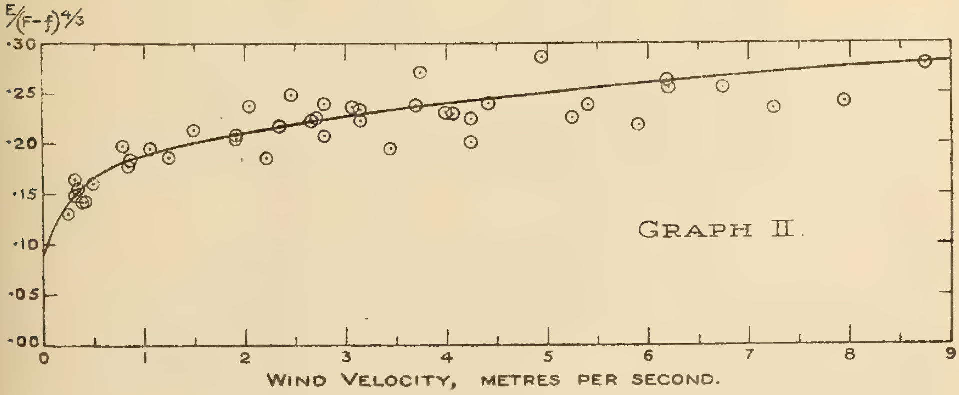
GRAPH I.

The cup anemometer is regarded as the more accurate instrument. The agreement with the readings of the cup anemometer is close enough to establish the claim that the kata-thermometer is an accurate anemometer.

It must be borne in mind that the kata must be shaded from the sun's rays, direct or reflected, when used as an anemometer. The kata shows the effect of eddies and air movements which are too small to affect cup or fan anemometers. This gives it an especial value in the investigation of the ventilation of rooms.

As shown in the paper cited above, there is a limiting velocity value beyond which the kata no longer follows the formula.

With respect to the observations detailed in Table 2, a certain amount of variation was found in the values obtained for the constant, the mean value as given by the graph being 0·49. This variation may be due to (*a*) experimental errors of observation, (*b*) errors due to causes the effect of which could not be determined. In this latter class would be included errors due to the fact that the kata, being a very sensitive instrument, detects a slight change in temperature or velocity before it is shown by thermometer or pressure gauge. Errors due to radiant heat, alteration of pressure, or to changes in the glass and liquid of the instrument must also be included in class (*b*). For example, it was found that the temperature of the water in which the kata was heated had a slight effect on the cooling power, this being greater when the water was at a temperature of 80° C. than when it was 45° C. Water in a Thermos flask at about 80° C. should be used for all kata observations.



GRAPH II.

In this connection it may be noted that the first of a series of observations of cooling of the kata is not reliable and should be neglected. Since errors under class (*a*) would be eliminated by taking the mean of a number of observations (as was done), it would appear that the variation in the constant must be due to undetermined causes. This is also pointed to by the fact that observations taken on any one day gave constant values.

If the kata is used to determine the cooling power under ordinary conditions of wind and temperature, the loss of heat is obtained by dividing the kata factor by the time of cooling, hence the formula $H/\theta = 0{\cdot}27 + 0{\cdot}49\sqrt{V}$ does not enter into the calculation. To deduce the wind velocity from the cooling power, however, the above formula is used, and an error not exceeding ±12 per cent. may occur in the result, this error being due to the variation of the constant discussed above. For example, if the velocity given by the kata is 2·5 metres per second, then the true velocity will lie between 2·2 and 2·8 metres per second. Similarly, in calculating kata cooling powers from temperature and wind velocity data published in the meteorological reports, errors of the same magnitude may come in.

Table 2.—Values Obtained from Experiments in Wind Tunnels.

H.	θ.	Velocity.	H/θ.
35·6	17·0	13·8	2·09
39·6	19·7	12·2	2·01
37·9	19·4	12·2	1·95
33·1	17·0	11·7	1·93
35·2	19·4	10·4	1·81
35·2	19·8	9·05	1·78
33·2	19·4	8·5	1·71
32·0	19·6	7·4	1·63
31·1	20·0	6·8	1·55
31·5	19·7	6·7	1·60
31·3	19·6	6·7	1·59
30·2	19·4	6·5	1·56
31·6	20·5	6·5	1·53
31·3	19·7	6·3	1·59
28·9	19·6	6·2	1·47
27·9	19·5	5·4	1·43
26·0	19·5	5·0	1·33
21·4	19·5	3·2	1·10
19·7	19·4	2·8	1·02

Part II.—*Evaporative Power in Moving Air.*

Having re-determined the formula for the dry kata in moving air, it became necessary to re-investigate the formula for the cooling of the wet kata in moving air, which was provisionally given in the Royal Society paper cited above.

It was not possible to use the large wind tunnels for any extensive investigation of the wet kata, because these tunnels are placed in very large halls, the temperature and humidity of the atmosphere of which we could not vary at will. After taking some preliminary observations in these tunnels, which gave us indications for the construction of the new formula, we employed a tube 4 feet long and 3 inches in diameter, and a fan which sucked air through the tube at velocities which could be varied at will. Two gauze screens were fixed in the tube to ensure an equable flow of air, and suitable side openings provided through which (1) the kata, (2) a dry bulb, (3) a wet bulb thermometer, were inserted in order, so that the kata came first and the wet bulb nearest the fan. The thermometers were read immediately before the kata was introduced in each observation of cooling rate.

The whole apparatus was placed in a small chamber, kindly placed at our use by Mr. H. R. Davis, at the works of Siebe, Gorman, Ltd., a chamber in which we could at will vary the temperature and humidity through fairly wide ranges. To determine the velocity of the wind we used the reading of the dry kata and our new formula $H/\theta = 0{\cdot}27+0{\cdot}49\sqrt{V}$, taking dry kata readings alternately with wet kata readings in each series of determinations.

To determine humidity we used the readings of the wet and dry bulb thermometers and the tables given by the Royal Meteorological Society. Our results are given in Table 3, and are plotted out in Graph II, and the formula which best fits these appears to be

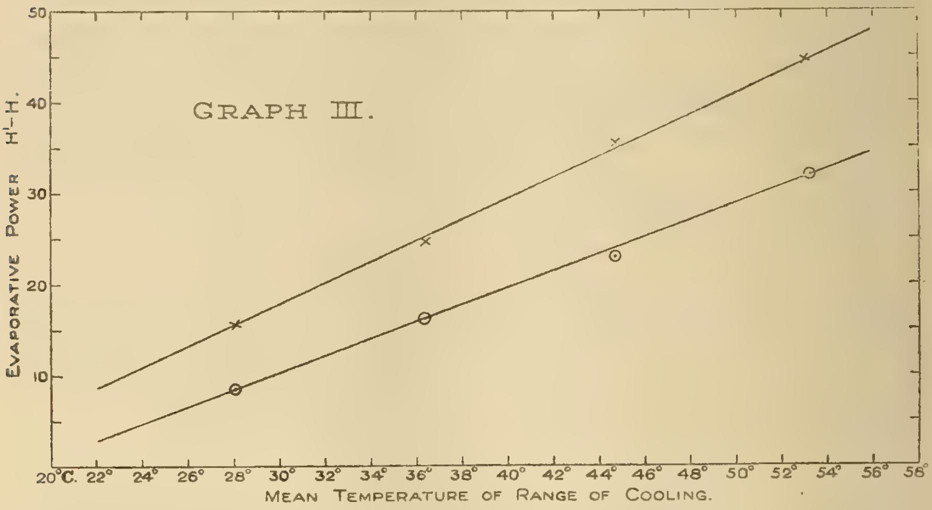
$$E/(F-f)^{4/3} = 0{\cdot}85+0{\cdot}102\,V^{0\cdot3},$$

where $E =$ the cooling power due to evaporation, $F =$ the vapour tension in mm. Hg. at 36·5° C. (the mean temperature of the kata surface), $f =$ the vapour tension of the air, and $V =$ the velocity of movement of the air in metres per sec. The full formula for the wet kata is then

$$H = (0{\cdot}27 + 0{\cdot}49\sqrt{V})\,\theta+(0{\cdot}85+0{\cdot}102\,V^{0\cdot3})\,(F-f)^{4/3}.$$

The method available to us only allows an approximate fit to be obtained, firstly, because the velocity is not measured at the same time as the cooling of the wet kata, but just before and after, and the electric power driving the fan may vary slightly; secondly, because the relatively sluggish thermometers may not indicate slight changes in air temperature and humidity, which the excessively sensitive wet kata responds to; thirdly, because the tables, from which the vapour tension is deduced from wet and dry bulb temperatures, are admittedly only approximately correct; fourthly, because of errors in time, the wet kata cooling very rapidly. Error might also arise if too thick a cover was used for the wet kata, and an excess of hot water retained around it. We avoided any such error by using a muslin finger stall. After warming in the Thermos flask, excess of water was removed by gripping the glove above the bulb and drawing it up tightly, and giving the kata one or two downward shakes. Using these means, successive readings of the wet kata gave closely concordant results.

We cannot claim that the formula allows us to determine correctly either *f* or V from wet kata readings, but it does allow us to determine approximately from known data of *f* and V given in meteorological records, what would be the evaporative cooling power. We only propose to use it for that purpose. Using in addition to the ordinary kata one calibrated from 90° to 75° F., and another from 110° to 130° F., we investigated the effect of the surface temperature on the evaporative power. Graph III gives the results we obtained in two of our experiments carried out at different air temperatures and vapour tensions. In one case the evaporative power increases from 7 to 34, a difference of 27. In the other case from 15·5 to 44·5, a difference of 29. The mean surface temperature increasing in each case from 28° to 53° C. It follows that not only in evaporation from the skin but in the drying of materials the surface temperature is of great importance. By means of the kata the evaporative power of drying chambers can be investigated and drying processes made exact in place of being empirical.



PART III.—*Effect of Barometric Pressure on the Cooling Power.*

In the Royal Society paper cited above, the question of the effect of barometric pressure on the cooling rate of the kata was briefly considered, and a formula expressing the effect put forward, viz.,

$$H = C\sqrt{p},$$

where p = the pressure and C is a constant at constant values of kata bulb and enclosure temperatures, and H applies only to cooling by convection.

Then $$\frac{\delta H}{H} = \frac{1}{2}\frac{\delta p}{p},$$

Table 3.

Temperature.		Dry kata. H.	36·5−t. θ.	H/θ.	Wind velocity. Metres per sec.	With kata. H′.	H′−H.	Vapour pressure. f.	F−f.	$\frac{H'-H}{(F-f)^{4/3}}$.
Wet bulb.	Dry bulb.									
10·8	14·6	37·7	21·9	1·72	8·76	73·3	35·6	7·6	38·0	0·279
10·9	14·6	36·2	21·9	1·65	7·93	66·9	30·7	7·7	37·9	0·241
10·7	14·4	35·2	22·1	1·59	7·26	65·3	30·1	7·7	37·9	0·236
21·4	23·8	19·6	12·7	1·54	6·73	41·9	22·3	17·1	28·5	0·256
22·9	25·2	16·9	11·3	1·49	6·20	37·9	21·0	18·8	26·8	0·262
10·7	14·4	33·0	22·1	1·49	6·20	65·3	32·3	7·7	37·9	0·255
17·0	19·6	24·6	16·9	1·46	5·90	47·6	23·0	12·7	32·9	0·218
20·2	25·6	12·9	10·9	1·41	5·41	36·9	24·0	13·8	31·8	0·238
10·7	14·4	30·7	22·1	1·39	5·23	59·5	28·8	7·7	37·9	0·226
18·2	25·5	15·0	11·0	1·36	4·95	46·9	31·9	11·3	34·3	0·286
24·6	27·0	12·3	9·5	1·30	4·42	29·6	17·3	20·8	24·8	0·239
10·7	14·4	28·5	22·1	1·28	4·25	59·5	28·8	7·7	37·9	0·226
13·3	16·6	25·5	19·9	1·28	4·25	49·5	24·0	9·4	36·2	0·206
23·4	26·0	12·6	10·0	1·26	4·08	31·1	18·5	19·2	26·4	0·231
18·5	22·7	17·2	13·8	1·25	4·00	41·3	24·1	12·7	32·9	0·229
17·2	25·5	13·4	11·0	1·22	3·76	44·8	31·4	10·2	35·4	0·270
13·7	20·4	19·5	16·1	1·21	3·68	48·6	29·1	8·2	37·4	0·238
16·0	18·9	20·7	17·6	1·18	3·45	42·4	21·7	11·5	34·1	0·196
18·2	22·3	16·1	14·2	1·14	3·15	40·7	24·6	12·7	32·9	0·233
15·5	21·0	17·6	15·5	1·14	3·15	43·8	26·2	10·0	35·6	0·223
18·8	26·0	11·8	10·5	1·12	3·08	40·5	28·7	11·0	34·6	0·255
17·2	25·5	11·9	11·0	1·09	2·80	39·7	27·8	10·2	35·4	0·239
16·7	20·2	17·7	16·3	1·09	2·80	40·8	23·1	11·3	34·3	0·207
23·7	26·4	10·9	10·1	1·08	2·73	28·4	17·5	19·4	26·2	0·225
24·2	26·6	10·6	9·9	1·07	2·67	26·9	16·3	20·5	25·1	0·222
19·5	22·0	15·1	14·5	1·04	2·47	39·0	23·9	14·9	30·7	0·249
13·8	20·5	16·4	16·0	1·02	2·34	43·8	27·4	8·2	37·4	0·218
13·4	16·6	19·9	19·9	1·00	2·22	42·1	22·2	9·4	36·2	0·184
19·2	27·4	8·8	9·1	0·97	2·04	34·3	25·5	11·6	34·0	0·237
24·1	25·5	10·4	11·0	0·95	1·93	25·2	14·8	21·1	24·5	0·208
18·8	23·0	12·8	13·5	0·95	1·93	33·8	21·0	13·5	32·1	0·206
15·5	21·1	13·4	15·4	0·87	1·50	38·2	24·8	10·1	35·5	0·213
13·4	17·2	15·7	19·3	0·82	1·26	38·2	22·5	9·3	36·3	0·187
18·9	26·0	8·2	10·5	0·78	1·08	29·5	21·3	12·0	33·6	0·196
15·8	21·4	11·0	15·1	0·73	0·88	32·4	21·4	10·2	35·4	0·184
14·2	20·8	11·4	15·7	0·72	0·84	33·6	22·2	8·5	37·1	0·179
20·0	29·0	5·3	7·5	0·71	0·81	26·7	21·4	11·9	33·7	0·197
21·5	24·3	7·6	12·2	0·62	0·51	21·7	14·1	17·0	28·6	0·161
21·3	23·9	7·4	12·6	0·59	0·43	20·2	12·8	16·4	29·2	0·142
20·7	23·4	7·6	13·1	0·58	0·40	21·6	14·0	16·0	29·6	0·142
20·0	22·8	7·7	13·7	0·56	0·35	21·6	13·9	16·5	29·1	0·155
12·0	15·7	11.4	20·8	0·55	0·33	28·3	16·9	8·5	33·1	0·159
16·0	22·0	8·0	14·5	0·55	0·33	25·1	17·1	10·1	35·5	0·148
18·6	22·0	7·5	14·5	0·52	0·26	20·7	13·2	13·7	31·9	0·130
19·6	22·4	7·3	14·1	0·52	0·26	19·8	12·5	14·8	30·8	0·130

where δH is the increment of heat loss due to a small change in pressure δp, that is to say, the percentage change in rate of heat loss is one half the percentage change in pressure.

We have further investigated this matter in chambers constructed for high pressure and for low pressure observations, into which we could go ourselves together with our apparatus, chambers kindly put at our use by Mr. R. H. Davis, of Messrs. Siebe, Gorman, Ltd., to whom we are much indebted for the help thus rendered.

Since the theory of the loss of heat by the kata by convection shows that the rate of heat loss is proportional to the square root of the density, and therefore of the pressure, other conditions remaining constant, we may write

$$H_c = C\sqrt{p}, \qquad \text{(i)}$$

where H_c = heat lost by convection, p = pressure, and C = a constant. Experimental evidence shows that at ordinary temperatures and pressures half the heat loss of the kata is due to convection and half to radiation Assuming this to be case if H is the total heat lost, H_c that lost by convection, and H_r that lost by radiation, we have, since $H = 0{\cdot}27\,\theta$ (as proved in the Royal Society paper already cited),

$$H_c = \frac{0{\cdot}27\theta}{2} \quad \text{(ii)} \qquad H_r = \frac{0{\cdot}27\theta}{2} \quad \text{(iii)}$$

also from (i)

$$\frac{H_{c_1}}{H_{c_2}} = \sqrt{\frac{p_1}{p_2}} \quad \text{or} \quad H_{c_2} = H_{c_1}\sqrt{\frac{p_2}{p_1}}; \qquad \text{(iv)}$$

substituting for H_{c_1} in (iv) from (ii),

$$H_{c_2} = \frac{0{\cdot}27\theta}{2}\sqrt{\frac{p_2}{p_1}}, \quad \text{(v)} \qquad H_{c_2} = \frac{H_1}{2}\sqrt{\frac{p_2}{p_1}}, \quad \text{(vi)}$$

where H_1 is the total heat lost at a pressure p_1.

Now the loss of heat by radiation will be unaltered by changes of pressure, therefore if H_2 is the total heat lost at a pressure P_2, H_{c_2} being the loss due to convection, then

$$H_2 = H_{r_2} + H_{c_2}, \qquad \text{(vii)}$$

where H_r is the heat lost by radiation at a pressure p_1; hence substituting in (vii) for H_{c_2} from (vi),

$$\begin{aligned} H_2 &= H_r + \frac{H_1}{2}\sqrt{\frac{p_2}{p_1}}, \\ &= \frac{H_1}{2} + \frac{H_1}{2}\sqrt{\frac{p_2}{p_1}}, \\ &= \frac{H_1}{2}\left(1 + \sqrt{\frac{p_2}{p_1}}\right). \qquad \text{(viii)} \end{aligned}$$

To test the accuracy of this formula, experiments were carried out in still air under conditions of increased and diminished pressure. In the former case the value of the pressure was obtained by a gauge reading in pounds, and was only approximate; in the case of diminished pressure a mercury gauge was used reading to tenths of a centimetre. The results obtained are shown in the Table given below. To allow for the variation of temperature which took place, each of the cooling powers H_1 is reduced to that cooling power H_2 which it would have been if the temperature had been 11·7° C., this being the temperature at which the normal pressure (764 mm. or 15 lbs.) reading was taken; the formula used to obtain H_2 being

$$H_2 = H_1 \frac{\theta_2}{\theta_1}.$$

The column H_2 (calculated) gives the values obtained by equation (viii) H_1 being found experimentally to be 6·94 at temperature 11·7° and pressure p_1 764 mm.

The agreement between the experimental and calculated values are close, and this agreement seems to confirm the experimental observation that half the rate of heat loss at ordinary temperature is due to radiation.

Cooling of Kata in Compressed and Rarefied Air.

Mean time of cooling in seconds.	Mean temperature.	Pressure.	H_1.	H_2 (experimental).	H_2 (calculated).
61·7	11·8	29 lbs.	8·10	8·15	8·29
63·8	10·8	22·5 ,,	7·84	7·57	7·74
72·1	11·7	764 mm.	6·94	6·94	6·94
81·1	12·7	572 ,,	6·17	6·42	6·47
88·3	12·9	425 ,,	5·66	5·95	6·05
95·1	13·4	335 ,,	5·26	5·65	5·77

The Influence of External Concentration on the Position of the Equilibrium attained in the Intake of Salts by Plant Cells.

By Walter Stiles, M.A., Lecturer in Botany in the University of Leeds, and Franklin Kidd, M.A., D.Sc., Fellow of St. John's College, Cambridge.

(Communicated by Prof. Bayliss, F.R.S. Received July 2, 1918.)

Introduction.

The intake and translocation of dissolved substances in the living plant present one of the outstanding problems of the physiology of nutrition, and one which has for a long time attracted the attention of plant physiologists.

We know the metallic or ash constituents of the plant are taken in by the root in the form of salts, and eventually find their way, in some form or another, to every part of the organism. What is the mechanism of their intake and of their translocation from cell to cell? Can the movement be explained as a simple diffusion phenomenon, or are adsorption phenomena concerned, or chemical combinations, or is the process still more complex? The work recorded in this paper forms a first instalment of an attempted analysis of these phenomena of the intake and movement of salts in the living plant.

The experimental work which has so far been performed bearing directly on our problem falls mainly into two well-defined groups. In one group the unit of experiment has been the whole living plant, and the methods employed have been those of pot-culture and particularly water-culture. Although this method of attack resulted in the discovery of the fundamental principles of plant nutrition, yet the results obtained by the walter-culture method as usually employed, although furnishing data in regard to the relationship between the constitution of a solution external to the root and the resultant growth, do not afford quantitative data as to the intake of salts. In the absence of these quantitative data general laws for the relations of electrolytes to living tissue have consequently not been formulated.

In the second group of experiments isolated cells or organs or pieces of cell-tissue form the unit of experiment. In these cases the experimental difficulties in obtaining quantitative data are considerably less than when the whole plant is dealt with, and such data are consequently accumulating. The work that forms the subject of this paper belongs to this second group, and in the experiments here recorded the parts of the plant with which we have dealt are storage organs. While it is true that the general tendency has been for the physiology of nutrition of plants to become more and more a biochemical study of organs, or a biochemical study of the cell, rather than a study of the mode of living of the plant, yet it is perfectly obvious that it is neither possible nor desirable always to use the whole plant as the experimental object, and so long as the conditions of experiment are borne in mind, experiments on isolated cells or tissues should yield results of value which supplement those obtained by experiments with the whole living plant.

In this paper we deal with the absorption by plant tissue of salts presented singly to the tissue, and especially with the position of the equilibrium attained in this intake of salt, and the influence of the concentration of salt both on the rate of absorption and on the position of the equilibrium. Although a number of different salts were employed, the influence of the nature of the salt on the rate of its absorption is only touched upon briefly here; this question forms the subject of another paper.

Method.

The essentials of the method used are as follows. A number of discs o tissue of uniform dimensions are immersed in the experimental liquid and the change in electrical conductivity of the latter measured. In this way an approximate value is obtained of the change in ionic concentration of the external solution. The experimental tissues employed were those of potato and carrot, but, for reasons which will be explained later, carrot was chiefly used.

In order to prepare the discs, cylinders of tissue were obtained by means of a cork-borer of the necessary diameter, and the cylinders cut into discs of the required thickness by means of a hand microtome. They were then washed in distilled water thoroughly, or in tap-water followed by distilled water, and finally, lightly dried between blotting-paper before being used for an experiment.

In each experiment 100 c.c. of solution were employed, in which were immersed 40 discs of a diameter of 1·8 cm. and a thickness of 1 mm. The experiments were all performed in triplicate, which gives sufficient accuracy, as the regularity of the results obtained indicates. In all series in which the results were compared, the whole of the discs used in the series were mixed together and the sets of 40 discs for each individual sample were then taken from the general stock. It has been shown previously (13) that by this means the error arising from inherent differences in different samples of tissue may be considerably reduced. Comparisons are only made between experiments carried out contemporaneously on discs cut at the same time and thoroughly mixed.

As it seems almost certain that the rates of absorption and exosmosis must be influenced by temperature, the experiments were all carried out in a thermostat at 20° C., and the conductivity measurements were made at the same temperature by means of Kohlrausch's method. A dipping electrode was used, which was placed direct into the experimental solutions.

A difficulty arises in such work owing to the formation of diffusion gradients between the body of the solution and the absorbing surface. The difficulty is to be overcome by breaking down the diffusion gradients over the absorbing surface. This can be effected by shaking the bottles, and accordingly this was done in our experiments. The shaker was of the usual trolley pattern and was worked by an electric motor.

Experimental Results.

(a) *Potassium, Sodium, and Calcium Chlorides* (*Carrot*).—The results obtained with carrot immersed in solutions of these salts in a number of concentrations ranging from N/5000 to N/10 are shown in Tables I to III. In each case a control in which the tissue was immersed in distilled water was subjected to the same conditions as the experimental solutions. It will be observed that in all cases, except those of the most dilute solutions used, the conductivity of the external solutions progressively decreases, and in the case of the weakest solutions of the various salts the rise in conductivity is less than in the case of distilled water. The results are shown graphically in fig. 1, where the initial conductivities are taken as zero. We may assume with propriety that the decrease in conductivity represents approximately the difference between the absorption of the salt by the tissue and exosmosis from the tissue. Hence the ordinates between the curves for distilled water and the salt solution can be assumed as approximately proportional to the actual amount of salt ions absorbed, or that at any rate they represent minimum values for absorption.*

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* Possible causes making for a fall of conductivity in the external salt solutions *not* due to absorption and which would therefore make the values obtained greater than the true numbers for absorption :—

(1) Reactions between the exudate and the external solutions by which non-ionised molecules are produced. The possibility of such reactions appears to be ruled out as a serious source of error when we have regard to the dilution of some of the solutions and the magnitude of the decreases with higher concentrations, and further when the results that were obtained when the discs were subsequently returned to distilled water are considered.

(2) The action of sugars and other non-ionised substances in the exudate in reducing

Table I.—Carrot in Potassium Chloride of different Concentrations.

Time in hours.	Change in electrical conductivity of external solution.				
	Distilled water.	N/5000.	N/500.	N/50.	N/10.
0·5			− 3	−167	− 610
6·0	+ 80	+ 58	− 48	−372	− 970
24·0	+145	+ 92	−196	−892	−1600
52·0	+196	+137	−223	−992	−1850

Table II.—Carrot in Sodium Chloride of different Concentrations.

Time in hours.	Change in electrical conductivity of external solution.				
	Distilled water.	N/5000.	N/500.	N/50.	N/10.
3·0	+36	+30	+ 18	− 113	− 560
34·0	+87	+57	−124	− 476	−1070
41·5	+66	+19	−287	− 885	−1580
48·0	+58	+ 2	−340	−1020	−1720

conductivity of the external solutions. The quantity of such substances which diffuses out of the cells is quite negligible in this regard.

(3) A decrease in exosmosis due to the action of the salt on the tissue. Even if the extreme and unlikely assumption is made that exosmosis is reduced to nothing in all cases, the results with carrot tissue would not in the main be affected. The evidence available, however, from work on balanced solutions and the antagonistic action of ions, points in the other direction towards an increase in the rate of exosmosis under the action of single salts. [See, for example, Stiles and Jörgensen (11).]

Possible causes rendering the values for the fall in conductivity of the external salt solutions, as compared with distilled water, minimum values for absorption :—

(1) An increased exosmosis due to the action of the salts on the tissues.

(2) An independent absorption of ions. It is quite clear that by the conductivity method we shall only be measuring the approximate absorption of the least absorbed ion of a salt. When one ion of a salt enters the tissue in excess of the other, its place must be taken in the external solution by some other ion, either H, or OH (Pantanelli), or an iron escaping from the tissue (Meurer and Nathansohn). Its excess absorption will therefore not be measured. The extent to which one ion may be absorbed in excess of the other is seen as the result of the work of Nathansohn (8), Meurer (3), and Pantanelli (9). Moreover, owing to differences in mobility of different ions, and changes in the degree of ionisation resulting from replacement of one ion by another, the conductivity can only give an approximate value of the absorption.

Table III.—Carrot in Calcium Chloride of different Concentrations.

Time in hours.	Change in electrical conductivity of external solution.				
	Distilled water.	N/5000.	N/500.	N/50.	N/10.
0·5			+ 3	− 71	−343
14·5	+64	+35	− 53	−145	−457
20·5	+86	+53	− 57	−125	−370
36·25	+60	+17	−105	−181	−503
42·5	+54	+ 8	−116	−195	−470

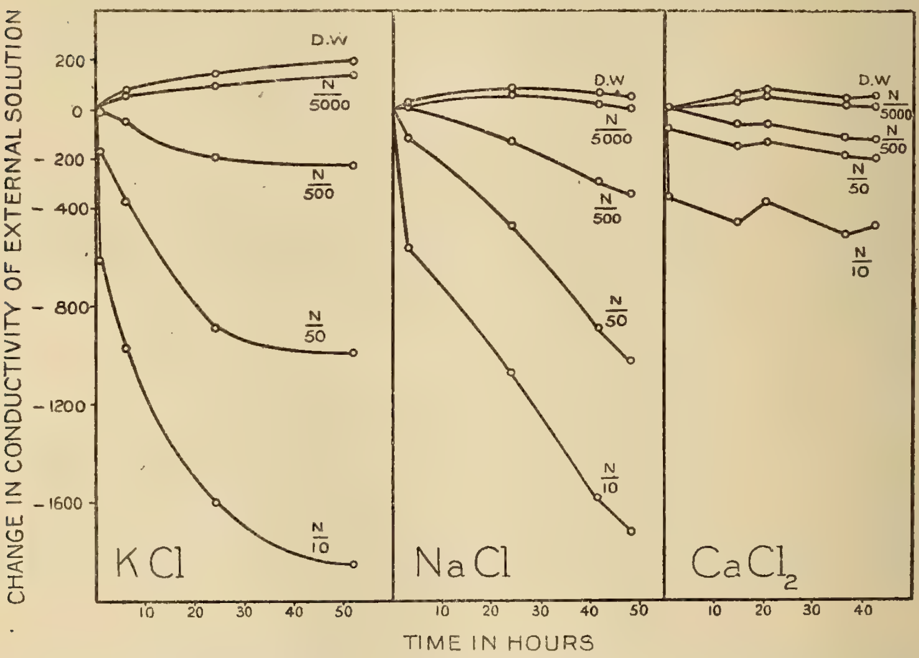

Fig. 1.—Changes in concentration of the external solution when carrot tissue is immersed in various chlorides of different concentrations.

We may note, then, first, that in all cases a decided absorption of salt takes place, and secondly, that the amounts of absorption increase along with increasing concentrations of salt in the exterior solutions. Thirdly, the rate of absorption is actually very slow at the temperatures used. In the case, for example, of the stronger concentrations of sodium and potassium chloride (N/50 and N/10) absorption is obviously not complete at the end of 48 hours. It is to be borne in mind that the discs are only 1 mm. thick. We shall return to this point later, in dealing with earlier work. Lastly, it is to be noted that the curves for the absorption from the calcium chloride solutions show that the uptake of calcium chloride is only about a quarter of that of sodium or potassium chloride from solutions of the same equivalent concentrations. The metal ion is, of course, less concentrated in the case of calcium chloride for solutions of equal normality, but this is insufficient to account for the difference in amount of absorption.

Table IV.—Absorption of Sodium Chloride by Potato Discs as Measured by Changes in Conductivity of External Solution.

Hours.	Observed.				Calculated putting distilled water = 0.			
	D. H_2O.	N/500.	N/50.	N/10.	D. H_2O.	N/500.	N/50.	N/10.
1·15	+ 69	+ 48	− 53	−550	0	− 21	−122	− 619
14·00	+306	+277	+215	−330	0	− 29	− 91	− 636
19·40	+452	+393	+300	−343	0	− 59	−152	− 795
38·10	+681	+620	+348	−367	0	− 61	−333	−1048
47·16	+746	+614	+298	−720	0	−132	−448	−1466
62·00	+666	+557	+287	−730	0	−109	−479	−1396
86·00	+656	+498	+207	−647	0	−158	−449	−1303

Discs washed 2½ hours, running tap water and five changes of distilled water.

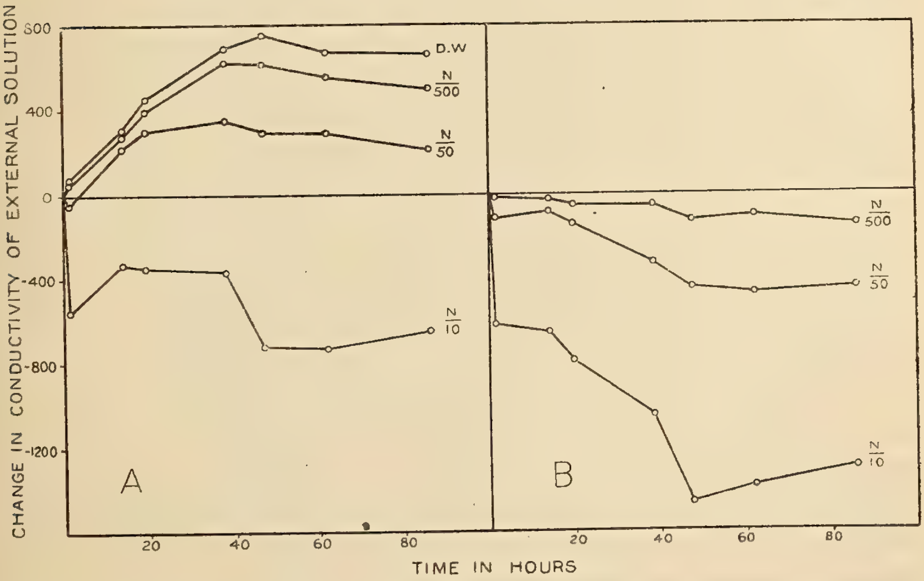

Fig. 2.—Absorption of sodium chloride by potato discs. A, changes in electrical conductivity actually measured; B, changes in conductivity relative to the change in the case of distilled water.

(b) *Sodium Chloride* (*Potato*).—The results obtained with potato tissue are complicated by the much greater exosmosis which occurs. Potato is also found to be less tolerant of the experimental conditions than carrot, and it is difficult to avoid injury and death of the discs due to secondary causes incident on the conditions of immersion. The differences between carrot and potato will be dealt with more fully in a subsequent paper, in which attention is centered on the phenomenon of exosmosis into distilled water in the case of various tissues. In the present research it has been found convenient to use carrot tissue mainly, and only a few experiments were conducted with potato.

The results of a series of experiments with sodium chloride are given in Table IV and in fig. 2. When the results are considered in the same way as those obtained with carrot tissue, that is, taking the initial conductivities as zero in all cases, and the curves representing the exosmosis into distilled water as a base line, the same conclusions appear. The amount of absorption increases with increasing concentrations of salt, and the rate of absorption is relatively slow.

(c) *Copper Sulphate* (*Carrot*).—We are here dealing with a highly toxic substance, and the results obtained (Table V and fig. 3) are of a different nature from those just described. The effect of the salt in all concentrations used is to kill the tissue by the end of the experiment. The action of the copper sulphate in all concentrations is greatly to increase the amount and rate of exosmosis from the beginning of the experiment onwards, the rate of exosmosis increasing in parallel with increasing concentrations of copper.* Exosmosis exceeds absorption in all cases. If, as we conclude, the action of the copper is very rapidly to destroy the organisation of the living cells and to render freely diffusible the full amount of contained electrolytes, this result is in accordance with expectation, as the internal concentration of salts in the tissue is equivalent to a conductivity of about 15,000, while the conductivy of the strongest solution of copper sulphate used amounts to only about 3200. The curves in fig. 3 also show that the increasing initial rate of exosmosis with increasing concentrations of copper sulphate is faster than the increasing rate of absorption of the copper sulphate. The final condition reached corresponds to an approximately equal distribution of all electrolytes (both tissue electrolyte and copper sulphate) throughout the whole system, *i.e.*, bathing liquid and tissue. This type of equilibrium condition is characteristic of dead tissue in contrast to living tissue, and is dealt with more fully in an ensuing section.

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* The type of exosmosis curve obtained in the action of a toxic substance upon tissue is fully dealt with in an earlier paper. See Stiles and Jörgensen (12).

The results obtained with potato are similar. Curves showing the action of copper sulphate on this tissue as well as on the roots of living bean plants are given in an earlier paper by Stiles and Jörgensen (11). They are similar to those given here for carrot.

Table V.—Carrot in Copper Sulphate of Various Concentrations.

Time in hours.	Increase in electrical conductivity.				
	Distilled water.	N/5000.	N/1000.	N/500.	N/50.
1·5	+ 26	+ 37	+ 61	+ 81	+102
18·0	+ 75	+ 66	+ 473	+ 674	+810
43·5	+121	+997	+1139	+1394	+833

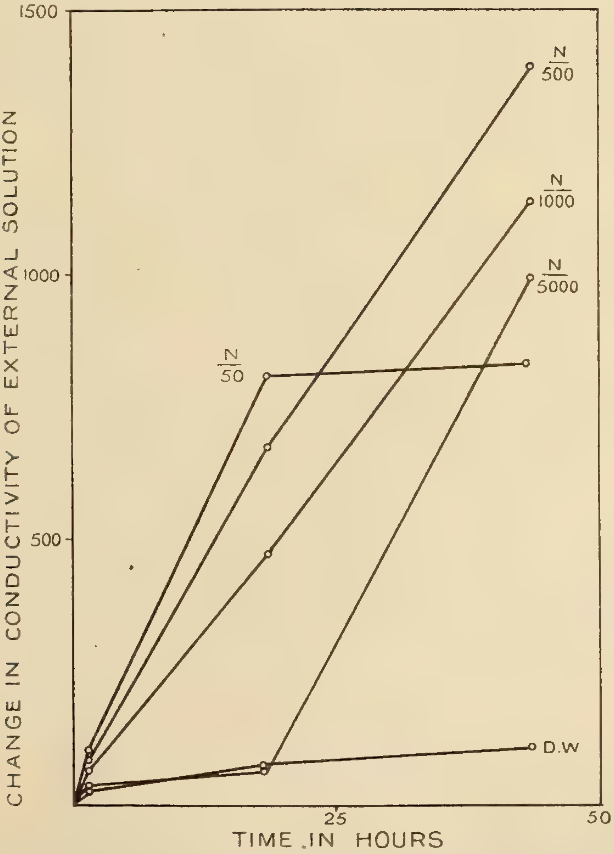

Fig. 3.—Changes in concentration of the external solution when carrot tissue is immersed in copper sulphate solutions of various concentrations.

(d) *Aluminium Sulphate* (*Carrot*).—With aluminium sulphate we are dealing with a substance which is not toxic, but which gives unusual results appearing at first sight similar to those obtained with toxic substances such as copper sulphate. In view of these results (Table VI and fig. 4), it is necessary to emphasize the marked absence of injurious action on the part of this salt even in comparison with such relatively harmless substances as potassium and sodium chlorides. If we take the more delicate potato tissue as a standard, it is very noticeable how perfectly healthy the discs remain in solutions of aluminium sulphate of all the strengths here used, in comparison with similar discs in distilled water, or in solutions of sodium or potassium chloride.

With aluminium sulphate an increase in conductivity occurred in all concentrations. The results, however, were irregular. The increase was less in N/5000 aluminium sulphate than in distilled water. It was greater in N/500, and greater again in N/50. The values in N/10, however, did not show a still further increase, but fell below those obtained in N/50. These irregularities do not appear to be due to experimental error, for exactly similar results were obtained on repeating the experiments. It is to be borne in mind also that all experiments were conducted in triplicate.

It is difficult to draw definite conclusions from these results until further analysis is made. For the present, our suggestion is that the aluminium ion is absorbed and its place taken by hydrogen ions or some other ion, which results in increasing the conductivity of the external solution. There are three lines of evidence which support this view. In the first place, as will be described in the second paper in this series, our experiments indicate that the sulphates of non-toxic metals such as potassium or sodium are very little absorbed in comparison with the chorides and nitrates. Since, as has been said above, our conductivity measurements only indicate the absorption of the least absorbed ion of a salt, this appears to show that the SO_4 ion is not easily absorbed. On the other hand, Meurer (3) has shown by direct analysis of the external solution that the aluminium ion is readily absorbed from aluminium sulphate solutions by carrot and other tissues. We quote his figures below.* Lastly, the exosmosis which occurs from the discs when subsequently returned to distilled water, as described in a succeeding section clearly indicates that absoption increasing with increasing concentrations has taken place from the aluminium sulphate solutions.

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* Meurer's results for the absorption of aluminium by potato are as follows (for the meaning of the expression absorption ratio, see at foot of next page) :—

375 grs. carrot in 750 c.c. of a 0·54 per cent. solution of $Al_2(SO_4)_3$.
Estimation of Al_2O_3 in 50 c.c. before experiment, 0·0800 grs.
After two days 0·0623, absorption ratio 0·62.
After four days 0·0600, absorption ratio 0·73.

Table VI.—Carrot in Aluminium Sulphate of various Concentrations.

Time.	Increase in electrical conductivity.				
	Distilled water.	N/5000.	N/500.	N/50.	N/10.
0·25				+ 93	+ 42
1·5	+ 57	+ 32	+112	+184	+130
12·42				+423	+345
14·5	+112	+109	+166	+405	
21·75	+ 98	+ 88	+159	+436	+375
38·75			+176	+590	+550
45	+110	+ 84	+136	+542	+520

Discs washed 28 hours, running tap water and five changes of distilled water.

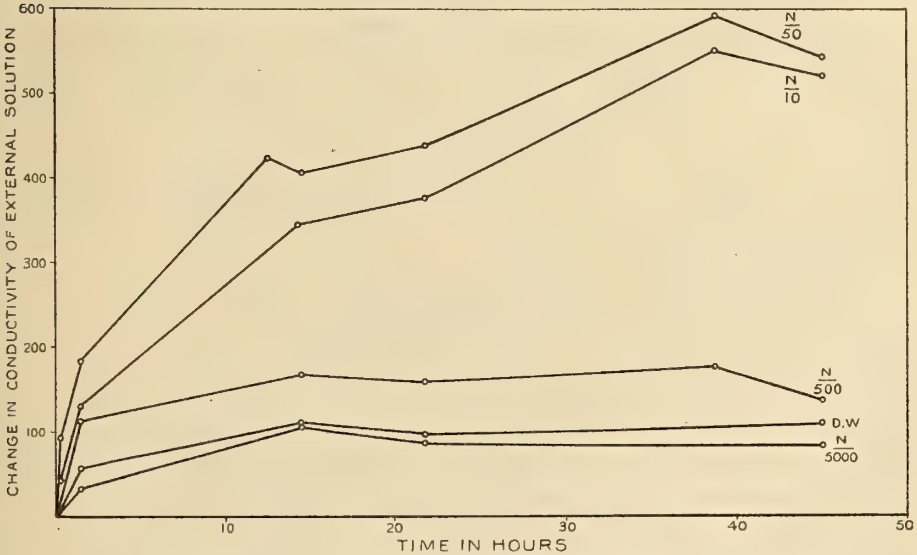

Fig. 4.—Change in electrical conductivity of external solution when carrot is immersed in aluminium sulphate of various concentrations.

375 grs. carrot in 750 c.c. of a 0·056 per cent. solution of $Al_2(SO_4)_3$.
Estimation of Al_2O_3 in 200 c.c. before experiment, 0·0337 grs.
After two days 0·0055, absorption ratio 11·33.
After four days 0·0038, absorption ratio 16·89.

Meurer's internal concentrations from which his absorption ratios are calculated are based, not on the tissue volume as in our case, but on the water content of the tissue. He concludes that the aluminium is principally absorbed by the cell-walls.

Concentration Equilibria reached in Salt Intake. "Heaping Up" of Salts in Living Tissue. Relation of External Concentration to Ratio of Internal to External Concentration at Equilibrium.

From the results described in the preceding section it is possible to obtain values for the amount of salt absorbed in any case per unit volume of tissue at any time. The values so obtained give us an expression for the "internal" concentration of the salt. Hence we can obtain the ratio of the concentration of the salt in the tissue to the concentration of the salt in the external solution.

The numbers are obtained on the assumption as before that the decrease in the conductivity of the external solution is a measure of the salt absorbed by the tissue. If the initial and final conductivities are C_1 and C_2 and X the exosmosis into distilled water, and if V is the volume of the external solution and v that of the tissue, then the ratio of the concentration of the salt in the tissue to the concentration of the salt in the external solution $= \frac{[(C_1+X)-C_2]\,V}{C_2 v}$. As, for the reason already discussed, C_2 is probably a higher value than actually represents the concentration of salt as compared with C_1 the number $\frac{[(C_1+X)-C_2]\,V}{C^2 v}$ is again a minimum value of the ratio $\frac{\text{internal concentration}}{\text{external concentration}}$. This ratio may be called the "absorption ratio."

Table VII gives the values of the absorption ratios at the end of each experiment for the different concentrations of the various salts used. It is not clear, as the graphical representations given above show, that equilibrium in absorption had been reached in all cases by this time. The curves having as ordinates the final internal concentration and as abscissæ the final external concentration, correspond to the equation $y = kc^m$, where y is the final internal concentration and c the final external concentration, and k and m are constants.

For this equation may be written in the form

$$\log y - m \log c = \log k,$$

and plotting log y against log c for our results, straight lines are obtained (figs. 5 and 6).

Although the actual values of these absorption ratios as precise measurements cannot be emphasized, the general conclusion which they indicate is remarkable.

Table VII.—Absorption Ratios in the case of Carrot and Certain Chlorides at the Approach of Equilibrium.

Initial external concentration in grm.-mols. per litre.	Relative concentrations.			Absorption ratios.
	Initial external.	Final external.	Final internal.	
KCl, 52 hours—				
N/5000	84	24	600	25·0
N/500	658	238	4,200	17·6
N/50	6,072	4,882	11,900	2·4
N/10	28,250	26,200	20,500	0·78
NaCl, 48 hours—				
N/5000	68	12	560	46·7
N/500	548	148	4,000	27·0
N/50	5,073	3,990	10,800	3·5
[N/50 (dead tissue)	5,073	5,060	4,450	0·88]
N/10	23,330	21,550	17,800	0·83
$CaCl_2$, 42·5 hours—				
N/5000	76	30	460	15·3
N/500	590	420	1,170	2·8
N/50	5,180	4,930	2,500	0·51
N/10	22,650	22,120	5,300	0·24

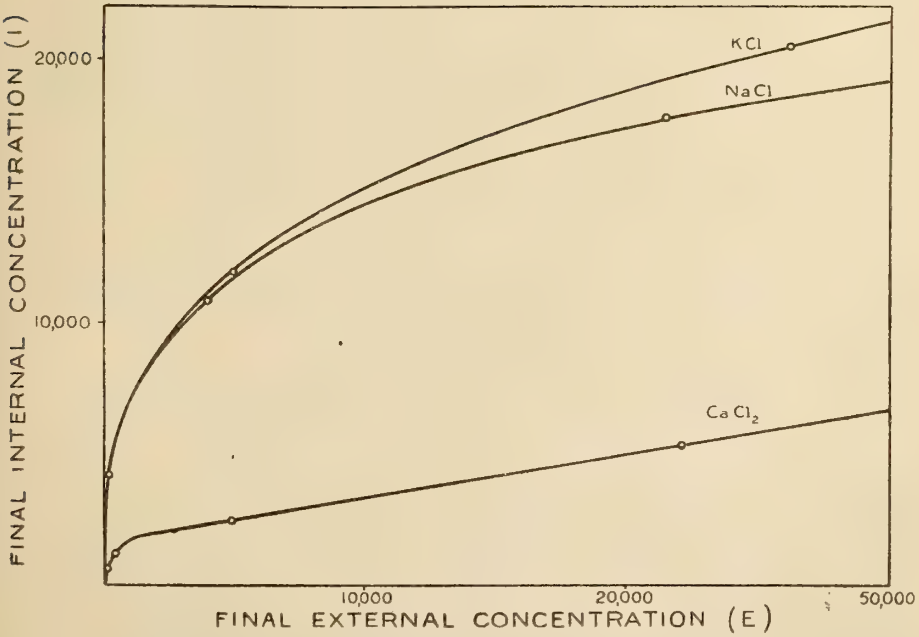

Fig. 5.—The relation between final external and final internal concentration in the case of carrot tissue immersed in certain chlorides.

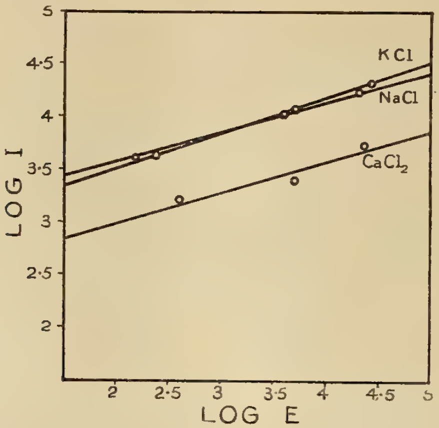

FIG. 6.—The relation between final external and final internal concentration in the case of carrot tissue immersed in certain chlorides. E is final external concentration and I final internal concentration.

It appears, in the first place, that the equilibrium reached in the absorption of a salt by living tissue depends upon the external concentration of the salt absorbed. While the *absolute amount* of absorption increases with increasing concentration of salt in the external solution as far as our experiments go, the amount relative to the external concentration nevertheless decreases rapidly from the lowest concentrations upwards.

Secondly, when the external concentrations of salt used are low, the absorption ratios become greater than unity, and with very dilute solutions probably rise to extraordinarily high values. For example, in the case of N/500 NaCl, the concentration of this salt inside the tissue after its absorption was 27 times as great as it was outside at the end of the experiment. Moreover, as we have already emphasized, this value is a minimum value for the equilibrium absorption ratio. A "heaping up" of salts is directly observed. With dead tissue, on the other hand, the equilibrium condition reached in absorption from similar solutions is one of approximately equal distribution of salt between tissues and bathing fluid.

As the concentration of the external solution is increased, the heaping up of the salt inside the tissue at equilibrium is reduced. In the highest concentrations used, *i.e.*, N/10, the degree of absorption required to produce nearly equal distribution of the salt throughout the tissue and the bathing fluid such as is characteristic in the case of dead tissue, is not reached. The absorption ratio at equilibrium is less than unity. With calcium chloride N/10, for example, where the absorption curve shows that equilibrium had been practically attained by the end of the experiment, the concentration then inside the tissue was still only about one quarter of that outside.

The calculated absorption ratios show very clearly that the degree of absorption depends to a large extent on the nature of the ions absorbed. Calcium ions are apparently far less absorbed than those of potassium or sodium.

Experiments in which at the equilibrium point reached in the intake of salts by living tissues of various kinds the absorption ratio is less than unity, that is, the final internal concentration is smaller than the external, have been previously recorded. From our results it appears that the failure to observe absorption ratios greater than unity has been due to the fact that the concentrations of the solutions used have been too high.* The results of previous workers are discussed in the following section.

Discussion of Previous Work. The Absorption Ratios found by Nathansohn and Meurer.

In his earlier work, Nathansohn (6) showed by means of chemical analyses of the external liquid and of the expressed sap that the marine alga *Codium*, in solutions of sodium nitrate, does not take up the nitrate ion to the extent required to produce equal concentrations inside and outside the tissue. The solutions he used were, however, relatively strong, varying from 0·5 per cent. to 5 per cent, that is in the region between N/20 and normal. We may quote a few of his results.

Table VIII.—Absorption Ratios in the case of Codium immersed in Sodium Nitrate. (Data from Nathansohn.)

External concentration.	Time.	Absorption ratio.
per cent.	days.	
0·5	4	0·56
0·5	10	0·68
0·5	2	0·58
1·0	5	0·44
1·0	5	0·49
3·8	2	0·43
4·8	2	0·41

Nathansohn remarked, however, that in sea-water, where the concentration of nitrate is low, the nitrate ion is often found heaped up inside the tissue, so that its concentration is greater inside than outside.

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* Nevertheless, it has often been observed that the concentration of a substance or ion inside the cell could be greater than its concentration in the bathing fluid. See *e.g.*, Moore and Roaf (4, 5) and Bayliss (1) for animal cells, Wodehouse (15) for plant cells (*Valonia*).

Later work by the same author, conducted on similar lines with slices of *Dahlia* tuber (7), and of beetroot and *Helianthus* tuber (8), show that with a variety of salts used in strong concentration the tissue never takes up the quantity of salt necessary for equality of concentration in the tissue and in the external liquid. This research contains further certain interesting indications that the absorption ratio varies in the manner shown by our experiments as described above. In a series of comparable experiments with *Dahlia* slices the following figures were obtained :—

Table IX.—Absorption Ratios with Dahlia. (Data from Nathansohn.)

Salt.	External concentration.	Time.	Absorption ratio (kation).
	per cent.	days.	
NH_4NO_3	1·5	4	0·32
	0·5	4	0·53
	1·0	2	0·63
	0·5	1	0·98
$NaNO_3$	1·0	6	0·51
	0·5	5	0·94
	1·0	4	0·59
	0·5	4	1·05

These results of Nathansohn's, although the author himself did not draw the conclusion, clearly show that the absorption ratio depends upon the external concentration in the manner indicated by our experiments, namely, that there is a rapid rise in the ratio as the external concentration of the absorbed salt is increased.

Nathansohn's work was later extended by Meurer (3), who used discs of beetroot and of carrot 3 mm. in thickness, immersed in a variety of solutions, The intake of salt, or rather of ions, was measured by the change in concentration of the external solution as determined by direct chemical analysis. Meurer's results confirmed those of Nathansohn in regard to the fact that equality of concentration was never reached. We may quote Meurer's figures obtained with carrot for comparison with those recorded in this paper for the same tissues. His results with beet are parallel.

It will be observed that in all cases the individual ions of the salt have not been absorbed to the extent required for equal distribution, even after four days, the absorption ratios being for the most part less than 0·5.

As in our experiments, Meurer also compared living and dead tissue. His results, quoted below, are the same as ours. He found that in the case of dead tissue the absorption ratio for both ions was approximately unity (actually slightly less). The failure to reach a condition of equal salt

Table X.—Ratio of Ion Concentration Inside Tissue to that Outside after Intake of Salt. (Data from Meurer.)

Salt.	Duration of experiment in days.	Concentration of external solution.	Absorption ratio.	
			Kation.	Anion.
K_2SO_4	4	N/17·5	0·402	—
KNO_3	4	N/20	0·524	0·570
$NaNO_3$	4	N/17	—	0·340
KCl	2	N/15	0·374	0·287
	4	N/15	0·548	0·386
NaCl	2	N/12	0·411	0·258
	4	N/12	0·489	0·307
$CaCl_2$	2	N/14	0·270	0·229
	4	N/14	0·282	0·270
	2	N/70	0·745	0·552
	4	N/70	0·846	0·852

distribution between strong solution and tissue appears as a property of living tissue in contrast to dead tissue, just as does the heaping up of salt absorbed from weak solutions.

Table XI.—Absorption of Magnesium Chloride by Living and Dead Tissue of Carrot. (Data from Meurer.)

Concentration of external solution initially.	State of tissue.	Absorption ratios.			
		After two days.		After four days.	
		Kation.	Anion.	Kation.	Anion.
N/24	Living	0·327	0·336	0·286	0·377
N/22	Dead	0·958	0·950	0·953	0·953
N/95	Living	0·563	0·774	0·577	0·895
N/105	Dead	—	0·950	—	0·869

When we compare Meurer's results with our own, it is to be observed that just as in the case of Nathansohn's work, the figures show distinctly that there is a greater absorption relative to the concentration with decreasing concentration of the external solution. Thus, with decrease in concentration from N/24 to N/95 in the case of magnesium chloride, the concentration of magnesium in the tissue relative to that outside rises, with an immersion time of 4 days in each case, from 0·336 to 0·774. Similarly, with calcium chloride, decreasing the concentration from N/14 to N/70 increases the absorption ratio for calcium from 0·282 to 0·846 after the same period of immersion.

Meurer's results, however, show no case of the heaping up of salt inside which takes place with further dilution. This is not surprising, as the concentrations he used all lie between N/10 and N/25, with the exception of one experiment with calcium chloride at N/70 and one with magnesium chloride at N/95. Our own experiments show that with calcium chloride a much greater dilution has to be reached in order to obtain heaping up than is required with potassium or sodium chlorides.

Influence of Thickness of Tissue Slices on Absorption Ratio.

The absorption ratios observed by Meurer for sodium and potassium chloride are less than those obtained by us, though the time of immersion was twice as long. This quantitative difference may possibly be due in part to the lower temperature at which Meurer worked (5° C.) as compared with that in our experiments (20° C.), but the chief reason is undoubtedly to be found in the thickness of the discs used by him. He worked with slices of tissue 3 mm. thick, while in our experiments the thickness of the discs was only 1 mm. Ruhland (10) has already emphasized the importance of the thickness of the tissue slices used in experimental work dealing with the absorption of salts by tissue from solutions. He compared the absorption by equal weights of 3 mm. and 1 mm. discs of beet in 0·4 per cent. calcium chloride and of carrot in 1 per cent. ammonium nitrate. With neither size of disc did the absorption ratio exceed unity, which agrees with our results for such concentrations, but within the time-limits of his experiments it is seen that the 1-mm. discs absorbed considerably more than the 3-mm. discs. The following Table summarises his results. After allowing for the thickness of the discs in Meurer's experiments, they fall wonderfully into line with our own.

It is clear that the relation between surface and volume of tissue in experiments dealing with the absorption of salts from solutions needs further examination, but as far as the relation between the absorption ratio and the concentration of the external solution goes, the solution of this question should not essentially modify the results described in the present paper.

Table XII.—Influence of the Thickness of the Tissue on the Absorption of Salt by Plant Tissue. (Data from Ruhland.)

Carrot seven days in 1 per cent. ammonium nitrate.

Thickness of tissue.	Absorption ratio.
3 mm.	0·5276
1 mm.	0·8342

Beet in 0·4 per cent. calcium chloride.

Thickness of tissue.	Kation.		Anion.	
	After two days.	After four days.	After two days.	After four days.
3 mm.	0·2582	0·3266	0·0354	0·0486
1 mm.	0·3421	0·5616	0·0522	0·0826

Exosmosis into Distilled Water after Immersion in Salt Solutions.

At the end of the experiments already recorded, the solutions were removed, the discs washed for a few minutes in distilled water, and then immersed in 100 c.c. of distilled water. Table XIII will serve as an example of the course of the exosmosis which results.

Table XIII.—Exosmosis into Distilled Water from Discs previously Immersed in various Concentrations of Sodium Chloride. (Rinsed quickly, once with tap water and once with distilled water.)

Time in hours.	Increase in electrical conductivity when previous solution was—				
	Distilled water.	N/5000.	N/500.	N/50.	N/10.
0·08	22	22	26	42	106
0·25	28	27	34	56	163
18·00	64	64	87	203	664
23·0	47	46	71	198	647
41·0	47	48	59	178	580
64·0	55	54	57	154	

The results show in all cases that the greater the concentration of salt in which the tissue is immersed the greater the exosmosis from the tissue on subsequent immersion in distilled water. Also, the amount of exosmosis does not increase to a constant and remain stationary, but after reaching a maximum value it slowly decreases as if electrolytes had re-entered the tissue. This phenomenon will be dealt with in a later communication.

In general, the results in regard to this exosmosis into distilled water corroborate the conclusions previously drawn as to the influence of concentration on the absorption ratio, for a similar relation is found after exosmosis into distilled water between external concentration and internal concentration, as after intake of salt.

Discussion.

From the experiments recorded in this paper, we may conclude that all the salts, or one or other of their constituent ions, readily enter cells of potato and carrot in all the concentrations employed. These two tissues show markedly different properties, exosmosis of electrolytes taking place in potato to such an extent as to mask the absorption when the electrical conductivity method is used, whereas in carrot this exosmosis is apparently negligible. For this reason, carrot is a much more suitable object for studying absorption of salts by the method employed than potato. Differences in regard to the water relations of these two tissues have been previously recorded (13), but it remains to be determined how far these different properties of the two tissues in regard to water absorption and salt exosmosis are correlated.

The substances employed fall broadly into two classes: those where the absorption does not produce any obvious injurious effect in the concentrations used, as in the case of sodium and potassium and calcium chlorides, and those where toxic action results as with copper sulphate. This division is, no doubt, arbitrary, for it is reasonable to suppose that these two classes are connected by a whole series of substances intermediate in their toxic action.

When toxic action takes place, this is accompanied by exosmosis in both potato and carrot, the initial rate of exosmosis being greater the greater the concentration of the external solution, as has previously been recorded (11).

In the case of carrot tissue immersed in solutions of potassium, sodium, and calcium chlorides, it is possible to follow the course of absorption by the electrical conductivity method. It may be said, in general, that the absorption is more rapid at first, especially for the first hour or two, and then gradually slows down until after 40 or 50 hours or more a condition of equilibrium is approached. The rapid absorption during the first minutes is particularly noticeable with the higher strengths of solutions (see the curves for N/10 solutions in fig. 1). In some recent work, Fitting (2) has concluded that the permeability of the epidermal cells of the leaf of *Rhœo discolor* as measured by the rate of entrance of potassium nitrate diminishes with time, or, in other words, that the rate of absorption of the salt decreases with time. His method of measurement of salt intake is based on the rate of deplasmolysis of the cells in solutions of the salt in question, so that only strong concentrations of the salt were employed. In a communication published while this paper was being written, Troendle (14) has recorded the results of some experiments on the absorption by bean roots and epidermal cells of *Acer platanoides* and *Salix babylonica* of salts in strong (hypertonic) solutions, the plasmolytic method being employed also here. Troendle's results are similar to Fitting's, but he states them more precisely. His conclusion is that the rate of absorption of the salt remains unaltered for the first few minutes (ten in the case of sodium and potassium chlorides), after which it falls off with time according to a logarithmic relation where the amount absorbed is proportional to the logarithm of the time. Troendle, on the basis of these results, puts forward the hypothesis that salt irritates the protoplasm which responds by transporting salt to its interior. This produces changes in the protoplasm which are of the nature of fatigue, and which increase with time according to the Weber-Fechner law.

That the absorption of salt should take place in proportion to the logarithm of the time seems to us a totally inadequate ground for putting forward such a definite theory of salt intake. Moreover, it is not at all clear to us that Troendle's conclusions as to the course of salt intake are justified. As far as we can follow the plasmolytic method of measuring salt absorption as described by Troendle, it would appear that any possible effect of the external concentration of the salt in influencing the rate of salt absorption is neglected, and each one of his curves appears, as far as we can judge from the data presented, to be constructed from numbers obtained with a whole range of external concentrations. The results we record in this paper show that under the conditions of our experiment the external concentration has a great influence on the rate of salt intake, and it ought certainly not to be assumed that external concentration is without influence on the rate of salt absorption although, of course, we do not know without experiment whether this is the case or not with hypertonic solutions and plasmolysed cells.

Considering now the influence of concentration on the absorption of salts, an examination of the figures in Tables I–III, and the curves in fig. 1, show that the initial rate of absorption is approximately proportional to the concentration of the external solution. This relation is, however, not long maintained owing to the proportionately greater accumulation of salt in the tissue in absorption from dilute solutions. From weak external solutions the salts not only freely enter the tissues but are accumulated there, so that the internal concentration is very much higher than the external. As the external concentrations are increased this heaping up of salt in the tissue at equilibrium is proportionately less until at a certain concentration no more than equal distribution inside and outside the tissue results, while in concentrations higher than this, concentration inside the tissue at equilibrium is less than that outside. Our results with these higher concentrations agree with those of Nathansohn and Meurer.

These facts show that the absorption of salts by the tissue used cannot be explained simply by diffusion. The results suggest that combinations take place between the salts used and some constituent or constituents of the living cells, so that either definite chemical compounds are produced, or adsorption compounds are formed.

The results obtained in regard to the influence of concentration on the absorption ratio conform to the adsorption equation. Obviously our results could be interpreted on the view that the intake of salts by the cell is simply an adsorption process, as advocated by Moore and his collaborators (4, 5), but it would also be possible to explain them on the basis of the formation of non-diffusible substances inside the cell in conjunction with changes in permeability of the cell which might result owing to the presence of the salt. Into a consideration of these questions we do not propose to enter further here. We may, however, point out that the behaviour of the treated tissues when removed from salt solution and put into distilled water suggests that the processes concerned in the intake of salts are to some extent reversible. In so transferring the treated tissues to distilled water, exosmosis occurs varying in amount according to the concentration of the solution previously used, and when the assumption is made that the exosmosis is mainly one of salt previously absorbed, it is found that the ratio of internal concentration to external concentration bears the same sort of relation to final external concentration as the absorption ratios. For any external concentration the ratio of internal to external concentration is now higher than that observed in the case of absorption ratios. These facts again make us hesitate to draw definite conclusions from the apparent conformity of the absorption results to the adsorption equation.

The influence of composition of the salt on its absorption will be dealt with in a further paper.

Summary.

1. The course of intake of salts by carrot and potato tissue has been followed by measuring the changes in conductivity of the solution of salt presented to the tissue. Concentrations of each salt were employed, varying from N/10 to N/5000.

2. In the case of copper sulphate, exosmosis exceeds absorption in all concentrations of copper sulphate. This is characteristic of toxic substances. The initial rate of exosmosis increases with increase of concentration of the toxic solution.

3. The exosmosis from carrot into distilled water is slight, while that from potato is considerable. For this reason carrot is a much more suitable subject for following absorption by the conductivity method than potato, where the absorption of salt is masked by the exosmosis of electrolytes from the tissue.

PROCEEDINGS
OF
THE ROYAL SOCIETY.

Series B. Vol. 90. No. B 633.

BIOLOGICAL SCIENCES.

CONTENTS.

Page

On the Four Visible Ingredients in Banded Bituminous Coal : Studies in the Composition of Coal, No. 1. By MARIE C. STOPES, D.Sc., Ph.D., Fellow and Lecturer in Palæobotany, University College, London. (Plates 11 and 12) 470

The Comparative Rate of Absorption of Various Salts by Plant Tissue. By WALTER STILES, M.A., Lecturer in Botany in the University of Leeds, and FRANKLIN KIDD, M.A., D.Sc., Fellow of St. John's College, Cambridge 487

Changes in the Teeth of the Guinea-pig, produced by a Scorbutic Diet. By S. S. ZILVA, Ph.D., M.Sc., and F. M. WELLS, Major, C.A.D.C. (Plate 13) 505

On a New Factor in the Mechanism of Bacterial Infection. By W. E. BULLOCK and W. CRAMER 513

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PRINTED AND PUBLISHED FOR THE ROYAL SOCIETY BY
HARRISON & SONS, 44—47, ST. MARTIN'S LANE, LONDON, W.C. 2.
Price Four Shillings and Sixpence.

No. B 633.

May 15, 1919.

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4. Carrot tissue absorbs potassium, sodium, and calcium chlorides in all concentrations examined. In the case of each salt, absorption is at first approximately proportional to the external concentration, but this relation is not continued with time, as the absorption progresses towards an equilibrium condition in which the ratio of internal to external concentration is not constant, but varies with the concentration. Similar results are obtained with potato.

5. The ratio of final internal to final external concentration has been called the absorption ratio. With low external concentrations it is many times unity, but with increasing concentration it diminishes, reaching with higher strengths of solutions a value considerably less than unity.

6. The relation between the final internal concentrations and the final external concentrations is given by the equation $y = kc^m$, where y is the final internal concentration and c the final external concentration. This is the adsorption equation, but the data presented are regarded as inadequate in themselves to justify the conclusion that absorption of salts by the cell is an adsorption process, and no proposals are put forward as to the mechanism of salt intake by the cell.

7. The results obtained are correlated with those of other workers on salt intake by plant tissue, especially those of Nathansohn, Meurer, and Ruhland in regard to salt intake as measured by direct chemical analysis, and those of Fitting and Troendle dealing with the absorption of salts from hypertonic solutions as studied by the plasmolytic method.

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On the Four Visible Ingredients in Banded Bituminous Coal: Studies in the Composition of Coal, No. 1.

By Marie C. Stopes, D.Sc., Ph.D., Fellow and Lecturer in Palæobotany University College, London.

(Communicated by Sir George Beilby, F.R.S. Received August 22, 1918.)

[Plates 11 and 12.]

Even after a century of investigation coal remains a complex mass of which the component parts can neither be handled nor separately identified. Many authors have recognised a variety of plant remains in coal, and the specific identification of these organisms and tissues has made good progress; but such work is truly palæontological, and the points of interest in it are the organisms and not the coal mass of which they form a part.

From another point of view coal is a rock, but, unlike most rocks, the nature and orientation of its component parts are scarcely known. One of the most distinguished of living geologists once said to me that he would like to have available about microscopic sections of coal rationalised data comparable with those already obtained by petrologists about thin rock sections. The present paper is a contribution in that direction. It is an attempt to present systematically certain observations made incidentally in the course of the joint researches Dr. R. V. Wheeler and I have been following out on various other aspects of the hydra-headed "coal-problem."

In this paper the only type of coal dealt with is British palæozoic (Coal Measure) streaky bituminous coal, the kind of coal which every household and most factories employ. The appearances of the microscopic sections of Cannels, Bogheads, Anthracites, and "Lignites" are each distinctive and significant, but they are not dealt with at all in the present paper.

The actual coal chiefly used in the research was the various bands of the Hamstead Colliery (Birmingham), and to Mr. L. Holland, the manager, and the Company, I am much indebted for facilities to collect the coal myself *in situ*, and for various courtesies; also to Mr. P. S. Lea I am indebted for carefully selected samples from the "Eight-feet seam" from West Cannock Colliery, S. Staffordshire. The observations were checked and supplemented by the examination of various other bituminous coals from widely separated localities.

In their text-book on the Petrology of Sedimentary Rocks, Hatch and Rastall (1913) describe ordinary coal as follows: "The humic or bituminous group includes the ordinary house, cooking, and steam coals, of which the appearance is so familiar as scarcely to need description. As a rule, they consist of a series of alternating bright and dull layers; in the latter only can remains of vegetable tissues sometimes be seen."

This may be taken as summarising a prevalent view; but, as will be seen on reference to p. 484 below, a conflict of opinion exists about the presence of plant tissues in the "dull" or the "bright" layers; some of the leading authors taking a view opposite to that of Hatch and Rastall, and stating that it is only in the "bright" layers that tissues are to be seen.

Preliminary Statement of the Present Contribution.

Essentially the present contribution to the subject consists in the explicit recognition not of mere "dull" and "bright" bands, but of *four* distinctive and visibly differing portions forming the mass of an ordinary bituminous coal; and the demonstration of the fact that these four portions can be recognised and separated from each other both macroscopically, by hand, and microscopically in thin sections; and that, further, these four portions react so differently to certain simple chemical treatments as to indicate that their chemical molecules should be substantially different from each other. Diagrams of these points, and of the relations of the four constituents to each other in an ordinary sample, as well as colour illustrations of the same in thin sections, are given.

These four distinguishable ingredients, all of which, in varying quantities, are to be found in most ordinary bituminous coals, I name provisionally as follows :—

(i) Fusain[*]	The equivalent of "mother of coal," "mineral charcoal," etc., of various authors.
(ii) Durain[†]	The equivalent of "dull" hard coal of various authors, the "Mattkohle" of Germans, etc.
(iii) Clarain[†]	Together the equivalent of "bright" or glance coal of various authors, the "Glanzkohle" of Germans. Sometimes the "bright" coal of an author seems to be the vitrain only.
(iv) Vitrain[†]	(Conchoidal fracture, brilliant in appearance.)

[These names, I am fully aware, do not represent chemical entities (with the possible exception of vitrain), but they do represent tangible entities of the same useful order as "jet," "granite," or "cheese."]

The generally "streaky" or banded nature of a seam of coal is of varying orders of magnitude, and as one magnifies a banded piece of coal more and more it becomes increasingly apparent how finely laminated it may be. Hence, a diagram of the arrangement of the bands natural size, magnified by 4, and another by 10 or even 20 diameters, may all show essentially similar lenticular intercalated lamellar structure.

In hand specimens sometimes bands two to four inches or more in thickness may be all "dull" coal, little, if at all, streaked with "bright;" and perhaps above or below that may be three or four inches of glossy "bright," only finely streaked with dull. Most coals, however, are more mixed than that, and the average "dull" band is from a quarter of an inch or more in thickness, and is all through visibly streaked with fine lenticels of "bright," while the "bright" portions are streaked with very variable bands of "dull." Both the "dull" and the "bright," both the fusain and the vitrain, are all essentially lenticular masses; these are often so horizontally extended and so thin that they create the impression of being fine horizontal bands. With very few exceptions they lie approximately parallel to the bedding plane of the deposit. The fusain is the least regular in its arrangement, but on the whole its more wedge-shaped portions tend to lie so as to be most apparent on the surfaces which are split parallel to the bedding. The appearance of fusain has very often been described.

---

[*] The French name, adopted into English by J. J. Stevenson (1911–13) and Stopes and Wheeler (1918), to replace our native unwieldy and misleading names "mother of coal" and "mineral charcoal."

[†] The first use of new terms suggested by the present author, and each based on a Latin root descriptive of the substance and terminated in *-ain* to match *fusain*. The latter word is a French word used by geologists in a specialised sense. It is based on the Latin *fusus*, and its application to the "mineral charcoal" came about in a circuitous way. The roots I have chosen are obviously and directly descriptive.

The fourth ingredient, the vitrain or brilliant bands, have been less considered in the past, but are, as a matter of fact, particularly interesting. These brilliant bands, in a favourable sample, are very définitely deliminated from the rest of the coal, much more so than are either the ordinary bright clarain or the dull durain from each other, which, owing to the finely lamellar nature of the coal in which they are interlarded, are sometimes hard to separate. The true vitrain, however, generally forms a very definite and often sharply straight-cut band, varying from 2 to 6 or 8 mm. thick. There are, of course, brilliant streaks of smaller size, down to almost hair-like flecks. The larger, however, are the more typical vitrain zones. They are notably less numerous and less in quantity in most coals than are the ordinary glossy bright clarain, and in some coals are scarcely to be found. They should be carefully distinguished from the very glossy almost vitreous thick zones of bright coal sometimes forming a great quantity of a seam, which, however bright, will always show streakiness however subdued. A true piece of pure vitrain is not streaky even with a magnifying glass.

In studying minutely the four different portions of a coal, one point should be borne in mind concerning the samples used; and that is, that they should all four be selected *in situ*, and as near to each other as possible. In the mine, therefore, blocks should be cut out, each showing all four ingredients as well differentiated and as nearly contiguous as possible. In this investigation I worked with well banded blocks about 6 by 8 inches cube. In the course of the work a large portion of each sample block is broken up, and the zones where the intercalation of the "dull" and bright is very fine, are useless for the isolation of the pure ingredients, as they cannot then be separated by hand. It must be recognised further that both durain and clarain can really never be got absolutely free from some streaks of each other, but if in the durain there are only few and hair-like streaks of clarain, and in the clarain only few and hair-like streaks of durain, they can serve for all practical purposes as pure enough to indicate the relative characteristics of really pure clarain and durain. With care, both fusain and vitrain can be separated by hand from favourable samples in a really pure state.

The Appearances of the Four Ingredients with the Naked Eye, i.e., *their Macroscopic Appearances.*

Fusain occurs chiefly as patches and wedges, somewhat flattened parallel to the bedding plane, and often with rather square-cut ends. It consists of powdery, readily detachable, somewhat fibrous strands. The orientation of the fibrous structure tends to be lengthways in relation to each wedge, and the various wedges on a bedding plane lie at various angles to each other, so that in any given light some appear dull and some glisten according to the direction the light catches the fibres.

The fusain is readily separated from the rest of the coal (which is all firmer than it in texture) by delicate scraping with a blunt knife, when the short, fibrous strands and small, sharp-pointed, irregular fragments fail freely on to a paper laid so as to catch them.

Where, as may happen, a thick wedge of fusain is contiguous with a true vitrain band, the fusain may appear as though embedded or sunk in the vitrain (*cf.* the text-fig., 2 *f*). The fusain can then be entirely scooped out, leaving exposed on the vitrain the hollow in which it lay, the surface of this vitrain hollow being curved and smooth. The contact surfaces of both clarain and durain with fusain, however, are much less precise, and an impression of the fibres of the fusain is generally left on the harder durain or clarain after all the friable detachable fibres of the fusain have been removed.

Durain occurs generally as bands of very variable thickness, and when seen in a face at right angles to the bedding plane, they appear parallel to it, though, if traced far enough, they generally reveal their ultimately lenticular shape. Wider bands of comparatively pure durain are less common, but bands, 2, 3, or more inches thick are often sharply differentiated from the adjacent streaky bright clarain.

Durain is hard, with a close, firm texture, which appears rather granular even to the naked eye. However straight the break across it, the broken face is never truly smooth, but, if looked at closely, always has a finely lumpy or matte surface (see Plate 11, fig. 1). Generally, even in the dullest of durain bands a few (or many) flecks or hair-like streaks of bright coal are to be seen.

The intercalation of narrow bands of durain and clarain tends to increase at the junction of the broad "dull" and the broad "bright" bands, so that there is no large surface of contact between them which is sharp cut and well defined, even the purest clarain and the purest durain tend to have ravelled edges, which interlock (see text-fig. 4, junction between *c* and *d*).

Clarain occurs generally as bands of very variable thickness, and when seen in a face at right angles to the bedding plane they appear parallel to it. Like durain bands, they are ultimately widely extended lenticular masses. Clarain, even when considerably streaked with durain, has a definite and smooth surface when broken at right angles to the bedding plane, and these faces have a pronounced gloss or shine. This surface lustre is seen to be inherently banded, as well as to have bands of fine durain intercalated between its own bands (see Plate 11, fig. 2).

Vitrain occurs as definite rather narrow bands, in some instances straighter and flatter than the other bands of coal, and in some instances more obviously lenticular. True brilliant vitrain bands are often markedly uniform in thickness for considerable distances, and are commonly from about 2 mm. to 3 or 4 up to 6 or 8 mm. thick, but are very seldom much more than 8 to 10 mm. thick. The limiting layer between the vitrain and the contiguous clarain or durain is generally sharply marked and is often clean-cut definite surface (see Plate 11, fig. 3). A single brilliant band does not exhibit the fine banding detectable even in the brightest of clarain, but is a coherent and uniform whole, brilliantly glossy, indeed vitreous, in its texture. The compact vitreous band may split up readily in the fingers to small cube-like segments, but more generally they break irregularly when forced, as with a penknife point, when the curved irregular faces have well-marked conchoidal fracture (see Plate 11, fig. 4). As was mentioned in connection with fusain, the contact-surfaces of vitrain with the other ingredients of coal tend generally to be well defined with a firm, hard, and glassy face.

Effects of the four Ingredients on the Photographic Plate.

The four ingredients are differentiated by their potency in making images of themselves directly (contact photos.) on sensitive plates.

Small pieces of approximately pure durain, clarain, and vitrain were placed on a negative, together with a little of the powdered fusain. All were taken from one sample.

By the method first described by Russell (*cf.* Russell, 1906, 1908, and used by Platt and Wheeler, 1913), a contact photo. was obtained.

The banded appearance of the contact photos. from blocks of ordinary streaky coal has been observed by the previous workers; the interest and novelty of the present photo. (for which I am indebted to Dr. Wheeler) lies in the very noticeable difference in intensity of the images made by the vitrain and the other parts of the coal. This can be seen on Plate 11, fig. 5, at A. That the brilliant vitrain should be the portion of the coal to make the most intense image on the plate is a point the significance of which is better discussed after some of the other characteristics of the four constituents have been considered.

Behaviour of the four Ingredients with certain Chemicals.

Work with a variety of chemicals is being undertaken, but the present paper is intended mainly to lead up to the microscopic distinctions between the four constituents, so that only two relevant chemical treatments will be noted here.

(i.) *With 10 per cent. KOH in Water + twice the Volume of 50 per cent. Alcohol* the behaviour of vitrain is interesting. Small pieces (about 3 × 5 or 4 × 6 mm.) of pure vitrain placed in this solution, without any previous treatment, and left in the cold, become slightly swollen and softened without the addition of any acid or any other chemical substance. In three or four days pieces in this solution acquire the consistency of hard cheese or soap, and with an ordinary razor thin flakes can be cut from them.* The vitrain alone, however, is affected in this way. The clarain largely breaks down when touched after being in the solution, and is hard and irregular to the razor's edge. The durain becomes so friable that any attempt to cut it with the razor breaks it down to a hard, gritty powder.

The penetrating power of alcohol should not be forgotten in considering the behaviour of coal to weak caustic solutions in it: a solution in water alone has not the same effect.

(ii.) *With strong Nitric Acid + a few Drops of Hydrofluoric Acid, followed later on by Neutralisation.*

Otherwise untreated samples of coal, of the three specified ingredients of coal other than fusain, placed in the mixture of acids, all tend after some days to break down to some extent, and the acid becomes tinged with brown. If left in the cold the pieces do not entirely disintegrate, but remain as smaller pieces. If after a week or so the acid is poured off, the pieces drained and then neutralised with strong potassium hydrate, they may still retain their solid nature. If the black solid mass is now placed at the bottom of a relatively large vessel, and water added in quantity, a proportion of each goes into solution as follows:—

Vitrain goes completely into solution if care is taken to select quite pure samples of vitrain. With the rapid addition of water there is at once formed a quantity of frothy "head," which ultimately, but very slowly, settles down. The "solution" looks quite black in bulk, but when it fills a thin tube and is held against the light, it is seen to be a clear tea-coloured liquid, containing no floating particles or suspended jelly-like precipitate. Exact measurements of quantities are not yet significant, but I found that 2 grm. of pure selected vitrain yield, with the addition of the necessary water, half a litre or more of a solution so strong that it looks coal-black in bulk. Where the vitrain is quite pure there is no undissolved *débris* at all; but if fine streaks are visible in part of the original material, a few small spores and fine *débris* may be found at the bottom of the flask.

---

* As a similar behaviour has been described for the run-of-mine *Lignites*, it should be remembered that the coals dealt with in the present paper are typical hard, true black, bituminous, palæozoic coals; and that one of the standard criteria of distinction between lignite and true black coals is the fact that aqueous solutions of potassium hydrate dissolve the former to some extent, with a brown solution, but do *not* affect the latter.

Clarain also goes largely into solution; the "head" of froth, however, is less in quantity and subsides more quickly. After standing, some *débris* settles at the bottom of the vessel, and this *débris* has, under the microscope, certain definite characteristics (see p. 478). The solution appears quite black in bulk and clear tea-coloured in a fine tube.

Durain forms no real froth, and the "solution" is rather paler than that of clarain, at first looking equally dark, owing to the number of very minute opaque particles suspended in it. After standing, the *débris* settles down and is seen to be considerable in quantity. Its microscopic appearance is described below (p. 478).

Fusain forms no froth and no true solution, the water generally remaining colourless, or being no more than straw coloured. The particles of *débris* are very heavy and numerous, settling more quickly than from the other coal ingredients (*cf.* p. 478).

In order to present these differences graphically, equal weights of each of the four coal constituents were taken, treated ultimately with equal amounts of water, allowed to stand overnight so that the *débris* settled, the clear solution then decanted off, leaving in each vessel 20 c.c., with the *débris*. Each vessel was then well shaken up, so that whatever was in each was mixed and held in suspension, and then from each a narrow tube was filled. These four tubes were standing vertically, when settled the contents presented the appearance shown in fig. 5, Plate 12 (coloured illustration).

The proportion of *débris* thus separated was much greater in the fusain tube than in the others; and in the tube the particles settled relatively quickly, and were big, black and opaque. The durain tube showed less *débris*, filling about one-third of the tube when the particles had settled, and the particles themselves were smaller, and not all opaque, but some were brown and translucent, while the solution was tea coloured, and in it were for long suspended fine amber coloured fragments of plant tissue.

The clarain tube showed still less *débris*, about one-sixth or less of the tube settled quickly, but for some time the finer generally amber coloured and clear particles of plant tissue, spores, etc., remained suspended in the clear solution, which was the colour of strong tea.

The true pure vitrain had no *débris*, save for an intrusive speck or two. The clear solution was strong tea coloured.

Micro-photos of the *débris* of the above experiments show some of their characteristic features. The three photos were all taken with identical illumination, magnification, and time exposure. The fusain *débris* (Plate 11, fig. 6), is almost entirely composed of angular, fibrous, sharp-pointed fragments of very varying size, black and opaque.

The durain *débris* (Plate 11, fig. 7) is largely composed of irregular but more polyhedrally shaped fragments, black and opaque, some of which have transparent edges; mixed with the opaque particles are clear, irregular fragments, which appear to be portions of the broken up walls of the macrospores. Scraps of cuticles, and so on, are sometimes seen, but are seldom recognisable. The opaque fragments generally preponderate in at least about the proportion of three to one.

The clarain *débris* (Plate 11, fig. 8) is much finer as a rule, and consists preponderatingly of clear, brown and amber coloured fragments of irregular size and shape. Mixed with these are some opaque black granules and fragments, possibly due to small inseparable streaks of durain. In the clear clarain *débris* can be recognised many cuticle fragments, pieces of or complete spores, and also oval or rounded particles which I have come to the provisional conclusion represent the *cell-contents* in a special condition, from which the surrounding cell walls have been dissolved away.

Clarain *débris* repays study with the high magnification, but for comparison with the other two *débris* it is shown in the figure on the same scale.

The Appearance of the Four Ingredients in Microscopic Sections of Untreated Coal.

A number of carefully selected blocks and small pieces were cut without any treatment save that necessary for sectioning by the grinding method. The coal is consequently quite unaltered (save for the permeating canada balsam mixtures which attach the sections to the glass) both in appearance and essential structure. The microscopic appearance of the four constituents are most noticeably different and characteristic, as will be apparent on reference to Plate 12, figs. 1–4 (coloured).

Before describing them in detail, I wish to say a word about coal microsections, many of which have been described by various authors. In several publications there are available for reference a number of excellent photographic reproductions of the structure and appearance of various coal sections. The reader should specially refer to Lomax (1911, 1915); White and Thiessen (1913); Jeffrey (1914, 1915); and Hickling (1917); and for critical references to the literature to Stopes and Wheeler (1918). All the illustrations above quoted show the finely stratified lamellar nature of the material in the coal sections, among which are recognised various plant tissues, resin bodies and other things in the coal.

Many carefully marked banded pieces of coal, and also small pieces composed of each separate ingredient as pure as could be obtained were cut into sections in the course of the present work, and from them the persistence of certain characteristics in each of the four types was made evident Describing the four ingredients now recognised in the order hitherto followed:—

The *fusain* (which, though friable, at times forms patches or lenticels in the coal, from which more or less complete sections can be cut) is almost black, opaque, and when it shows the cellular structure of the wood from which it was formed, it reveals the walls as much thickened and the cell lumina as being generally empty. When the section is approximately at right angles to the direction of the wood fibre, an appearance as shown in Plate 12, fig. 1 (coloured), results. This illustration, though in natural colours, looks almost black and white, save at *s* where is the gleam of an adjacent spore, showing the colour contrast between fusain and the other portions of the coal in section.

The botanical nature of the various plant fragments sometimes identifiable in the fusain does not concern us here; their general *optical* effect varies but little whatever species they are.

The *durain* being firm and hard in texture is more easily cut and ground than fusain, but as it tends to be rather granular, it is more difficult to finish finely than the more coherent and softer clarain. Sections show a granular matrix of roundish or polyhedral fragments, the majority of which are blackish and opaque. The granules are closely packed and form a coherent mass, but mixed with them are the most characteristic spore exines. These may be whole or in fragments. The macrospores are most conspicuous, and their very thick exines are clear and brilliantly coloured, almost red, though when thinner they are reddish gold to pale gold or amber colour. In durain the ground mass of rather opaque granules, and the large clear macrospore exines tend to preponderate, see Plate 12, fig. 2 (coloured). There may or may not be a number of small microspores mingled with the granules forming the bulk of the durain. Throughout the texture of the less pure, streaked durain are seen in section small, clear, generally lenticular bands or flecks of a more golden colour. These are the streaks of clarain which so commonly lie interbedded with the durain (*cf.* p. 474). The purer the durain the fewer of these clear patches are to be seen in the section. These should be distinguished from certain other light coloured bodies sometimes to be seen in the durain, viz., the supposed "resin" bodies and other small distinctive granules. These, though sometimes locally abundant, are less characteristic of durain than of clarain. One may say that, on the whole, durain is essentially composed of a high proportion of opaque, fine granules, with many macro- and microspore exines scattered through it like currants in a pre-war pudding. Even in a small streak of durain the spore exines tend to be flattened and oriented so as to lie parallel to the general bedding of the coal seam.

The *clarain* is the easiest portion of the coal to cut into good sections, and is the most interesting to the palæobotanist, for in the clarain lie the greatest variety of recognisable plant tissues and structures. Clarain is essentially clear, as contrasted with the opacity of durain. There may be in it clear bands and zones showing much disintegrated plant substance, also bands of clear cuticle, spore exines, "resin-bodies," and other structures of various shades from pale yellow to a rich reddish-amber, the great majority of which are translucent or semi-translucent (though among them may be some opaque granules and particles), and among this variety of material plant stem tissues, leaf-tissues and so on may be preserved and may even fill the whole area of the sections. Plant tissues so preserved are also essentially translucent, though colour contrasts of the various minute structures present make the cellular tissue evident. There are also, of course (see p. 475), the opaque streaks of durain which are common in clarain, and should be looked on as an impurity in it. As a general rule, one may say of clarain that it is essentially translucent in thin section, and the purer the clarain the more are all its components of some degree of translucency [see Plate 12, fig. 3 (coloured)]. Even in a small area of clarain, that forming a fine bright streak in a dull piece of durain, for instance, the arrangements of the materials in it tends to be parallel to the general bedding of the coal seam. Comparison of a number of sections with the various published illustrations makes it clear that clarain is the happy hunting ground of the palæontologist in search of preserved remains of the tissues composing coal, and fortunate it is for him that so large a proportion of most ordinary seams are more or less pure clarain. In it remains of all kinds are to be found, ranging from very small fragments up to preserved stem tissues running for inches unbroken in the coal. It appears that the richer detail and variety of structures in the clarain, more or less pure, have attracted most recent workers, and have formed the basis of the great majority of the good illustrations hitherto published on the micro-structures in coal.

Vitrain is, in my experience, unobtainable pure in *large* sections, as true vitrain occurs almost entirely in thin bands, which tend to break into small segments. In section, when pure, its essential characters are its translucency (in which it resembles clarain) and its structureless and uniform texture, in which it differs from all other parts of coal. As it is technically difficult (my own cutter and Mr. Lomax, to whom I sent samples, find it impossible) to grind down this substance to absolute uniform thickness over the whole area of the section, the section has areas shading from pale gold to ruddy brown, but these obviously depend on the varying thickness of the slice examined: the mass is, uniform in its structureless nature. Plate 12, fig. 4 (coloured), shows the yellowish to dark amber colour of the uniform mass. Scratches show up on the surface very annoyingly, and are due to minute irregularities even in the finest polishing stone; they are unduly conspicuous in photos., but when the eye examines a number of sections it readily detects the essential uniformity of the vitrain, and its structureless nature, as of a hardened glue or jelly. In it may be seen an occasional isolated spore, or a fine streak of durain may have been included, but if the purest, most brilliant vitrain is selected, it is essentially homogeneous. The illustration given on Plate 12, fig. 4 (coloured), illustrates this, though imperfectly, and offers a contrast to the standard sections of durain and clarain (Plate 12, figs. 2 and 3).

There is, consequently, in *pure* vitrain no banding or differentiation of parts in relation to the bedding plane of the deposit, though any individual mass of vitrain generally itself forms a horizontally extended band, lying parallel to the bedding of the coal.

The original thesis of this paper is borne out in the above details of these very various observations, and I think we may now see in ordinary bituminous banded coal four recognisably distinct and differentiable ingredients, for which I propose the names fusain, durain, clarain, and vitrain. These four, though difficult to separate completely, and ever tending to be interbanded and to penetrate each other, can yet, in most ordinary banded seams, be recognised by the naked eye, locally pure, and obtained by hand separation nearly pure. Such separated samples from a few adjacent inches of coal show marked differences: (i) in their effect on sensitive plates, (ii) in their behaviour with various solutions, (iii) in the quantity and character of the *débris* they yield under treatment, (iv) in the microscopic details of this *débris*, (v) in the microscopic appearance of the substances in thin, ground, untreated sections. Further, in their chemical analyses, distillation products, and so on they differ; but these features will be dealt with by Dr. Wheeler.

The above data apply particularly to the well-banded, relatively undisturbed coals of the Midlands, of which the Hamstead Colliery yields excellent examples. In some other seams, particularly those visibly affected by earth movements, the whole of the coal may have been slightly dislocated and altered in minute steps, which, though not affecting either the bedding of the roof and seam, or the coherence of the seam, may yet have destroyed the interbanding of the four ingredients, as described above. Such coal may all look very "bright," and may have only the streaks of fusain to represent the "dull." An example of such a coal is seen in part of the Pentre seam of South Wales, in which one might search for long before finding a block with anything but "bright" and fusain in it.

Naturally, in order to obtain any light on the characters of these four constituents, my endeavour was to obtain samples which contained the ingredients in layers of sufficient size and purity for the respective substances to be dealt with nearly pure. In most banded bituminous coals such samples can be found if sought for, though they may be insufficient in bulk to handle easily. The coal in bulk is generally composed of masses more intermingled, so that small bands or lenticels of one or the other ingredient are interbedded, and only separable by hand with great labour.

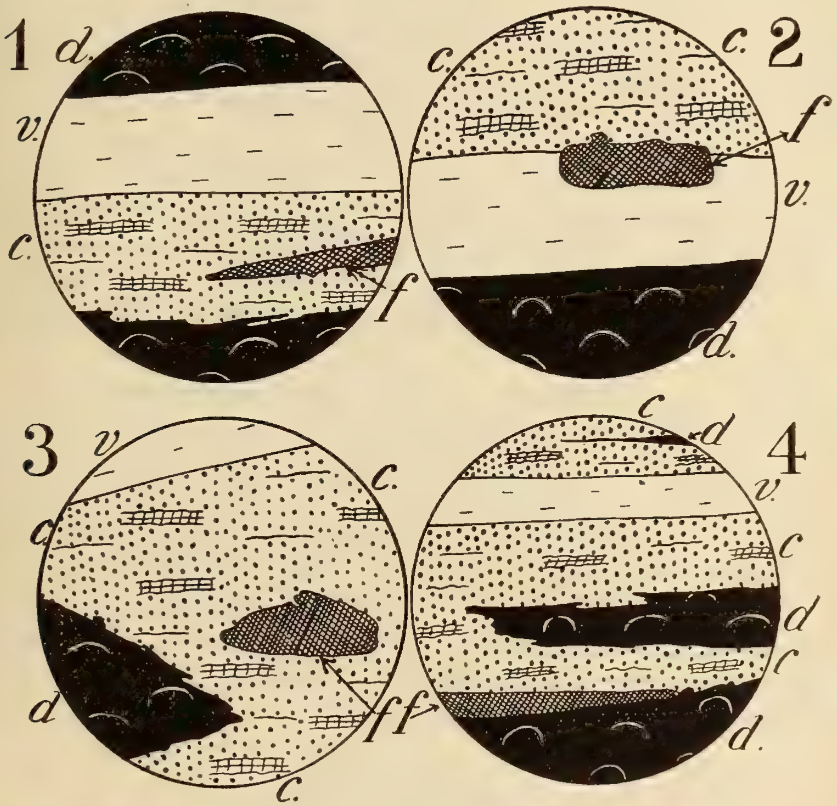
In general, therefore, sections of coal which have formed the subject of the investigations of previous workers will be found to contain at least two, and probably more, of the four constituents so laboriously separated in the present work.

It may be useful, and is certainly in keeping with the attempt to obtain a parallel to petrological knowledge, to give in a clear diagrammatic form the orientation of the ingredients generally to be observed. The accompanying four diagrams in the text (text-figs. 1–4) built up of conventional symbols representing each of the four above-named ingredients, illustrate the kind of distribution of the ingredients likely to occur in sections, taken without any special selection, from an average finely banded piece of bituminous coal.

These diagrams actually represent a low scale of magnification, four diameters, but, owing to the laminated nature of the coal, were more finely laminated regions taken and multiplied five or even ten times this, it would be possible to find areas which could justly be represented by the same diagrams.

An interesting feature to note is the comparatively straight line of contact between vitrain and either clarain or durain adjacent to it, when cut at right angles to the bedding plane; while the contact surfaces between clarain and durain tend to interlock in fine laminæ. Fusain tends to form irregular patches and wedges or lenses, which may have very small jutting projections into either the durain or clarain (see text-fig.) and in the vitrain may lie sunk in a comparatively smooth hollow (text-fig. 2).

As these four ingredients of coal which I provisionally delineate are none of them (with the possible exception of vitrain) actually homogeneous, nor are chemical molecular units, they do not even approximately represent the crystals in a petrological section of a rock; nevertheless, when represented on a low scale of magnification and in diagrammatic fashion, it may not be devoid of suggestiveness to compare such sections with those so useful to petrologists.



Text-figs. 1–4.—Diagrams of micro-photographs of sections of "streaky" bituminous coal, indicating the characteristic distribution of the four ingredients: *c*, clarain; *d*, durain; *f*, fusain; *v*, vitrain.

I hesitate to elaborate the matter at this early stage, but it seems possible that we have here a first step in the building up of an exact knowledge of the "physiography of coal sections," though the nature of the case debars the development of the theme from following quite the lines of the "microscopical physiography of minerals" laid down by Rosenbusch.

While the present recognition of four distinct ingredients in coal is, so far as I am aware, new, many authors have noted the banded appearance of coal, and particular attention has been paid to the "dull" and "bright" zones. The early and most excellent paper of Karsten (1826), in which the "matt" and "glanz" coal layers were not only described but correlated with preliminary chemical examinations, has been followed up by Rogers (1843); Dawson (1859, 1866, 1871); Grand 'Eury (1882); Renault (1882); Von Gümbel (1834); Wethered (1885); Gresley (1892); Seyler (1907); Barsch (1908); Stevenson (1911); Dowling (1911); Grout (1911); Lomax (1911); Pringle (1911); White and Thiessen (1913); Hatch and Rastall (1913); Grummitt and Hickling (1914); Jeffrey (1914); Lomax (1914, 1915); Strahan and Pollard (1915), as well as by many references of minor importance.

In these statements there is much difference of opinion, some authors stating that plant structure is found only in the "dull," others that it is found only in the "bright" coal. A detailed consideration of the views of Dowling (1911), White and Thiessen (1913), and Hickling (1917) is essential, but must be postponed to a later paper, when I hope to go into the matter more fully.

The lack of all preserved structure in "bright" coal maintained by various authors, and its jelly-like nature, suggested by Dowling, for instance, in my opinion indicate that the term "bright," as previously used, has covered both the bright clarain and the brilliant vitrain, differentiated in the present work, and also the secondary "brightness" resulting from natural agencies acting on a coal like the Pentre. So that in the past some authors have meant by "bright" the structureless brilliant vitrain, while others by the same word have meant the bright clarain which so often is full of plant structure; hence has arisen the directly conflicting statements about the presence of plant structure in "bright" and other coal.

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DESCRIPTION OF PLATES.

Plate 11—The plain Plate.

Fig. 1.—Photograph of natural broken surface of durain. At right angles to the bedding plane. Natural size.

Fig. 2.—Photograph of natural broken surface of clarain. At right angles to the bedding plane. Natural size.

Fig. 3.—Photograph of natural broken surface of small block of banded clarain in the centre of which is a broad definite band of vitrain. Note: In the photograph, owing to its brilliantly refractive nature, the vitrain shows as a broad white band. At right angles to the bedding plane. Natural size.

Fig. 4.—Broken surface of vitrain showing irregular conchoidal fractures. This was split parallel to the bedding plane. Natural size.

Fig. 5.—Contact photo. made by placing A, durain, B, clarain, C, vitrain, on a negative and enclosing them in a darkened chamber. Notice that the image obtained thus directly from the various ingredients is much stronger from the vitrain than from the others.

Figs. 6, 7, and 8 are all of micro-photographs of the *débris* obtained by treating the three ingredients by the method described on page 477. The three photographs are all taken on the same scale of magnification and with the same time exposure.

Fig. 6.—*Débris* of fusain. Note the sharp angular shape and the solid black appearance of the fragments.

Fig. 7.—*Débris* of durain. Note the more polyhedral shape of the black fragments and the presence of a number of less opaque ones.

Fig. 8.—*Débris* of clarain. Note the small number of black fragments and the high proportion of clear or nearly transparent fragments. Just above the centre several spores can be seen. Note also the much smaller size of the fragments from this material than from the other two.

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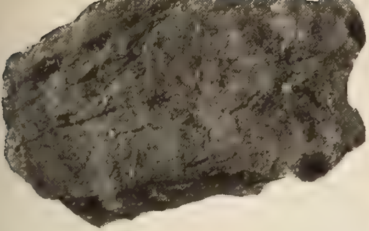

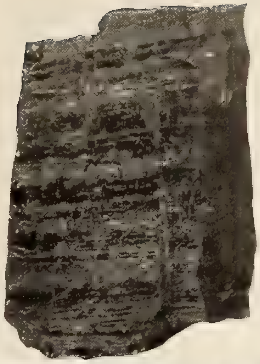

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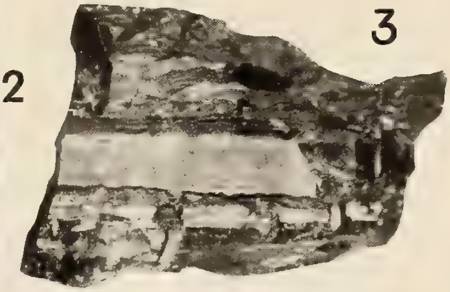

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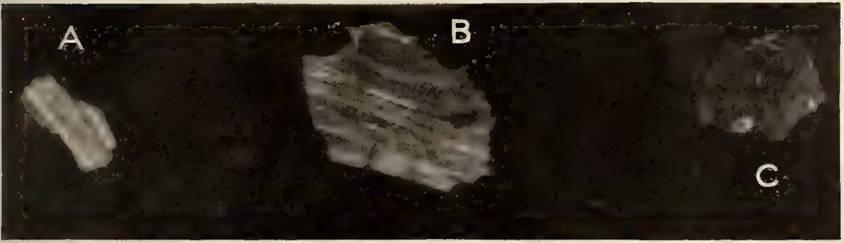

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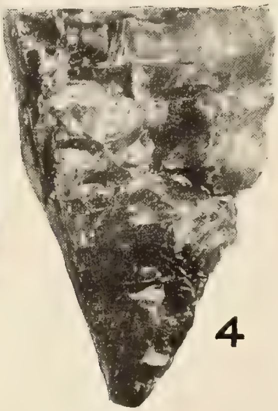

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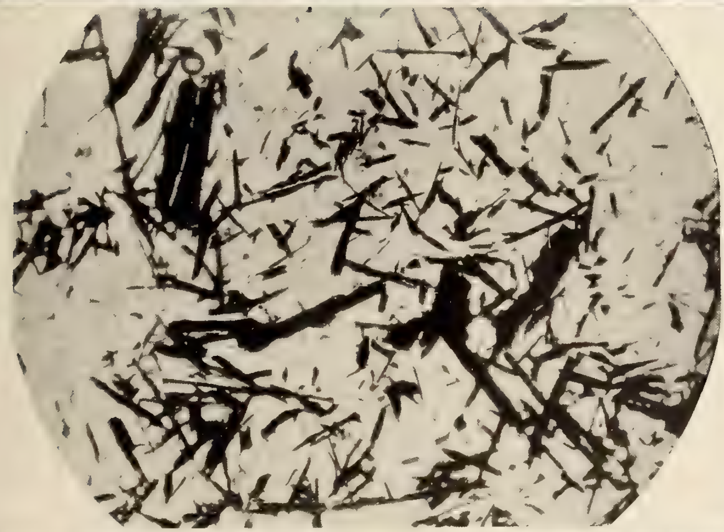

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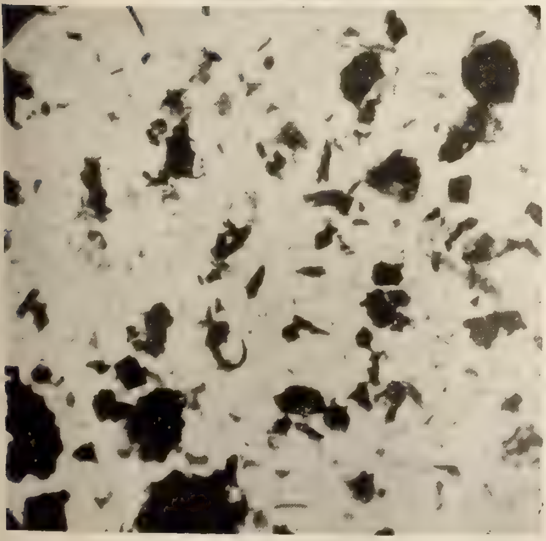

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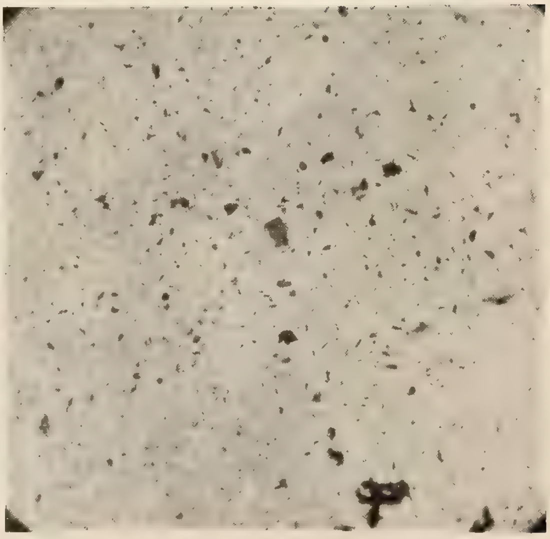

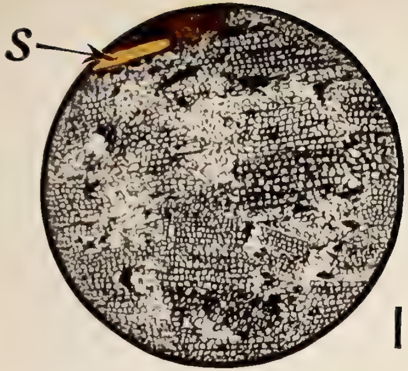

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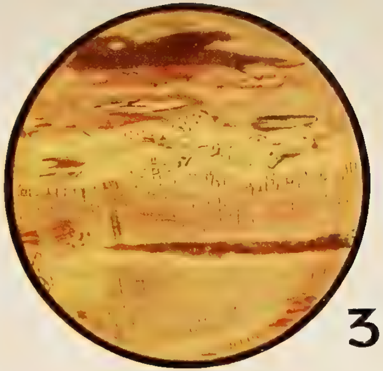

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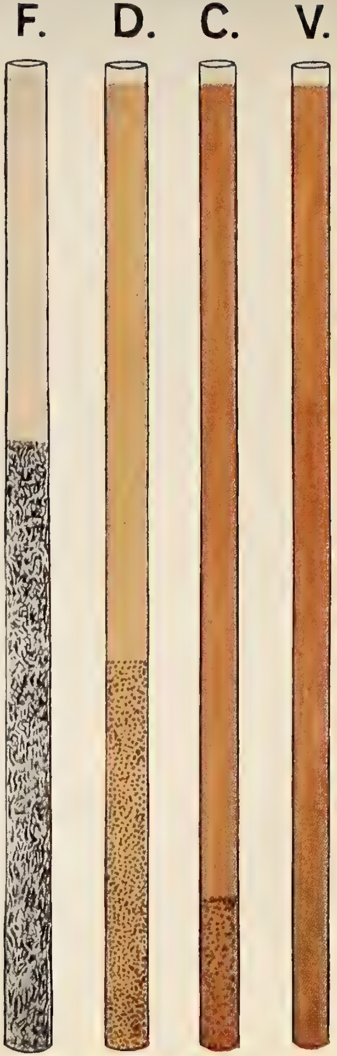

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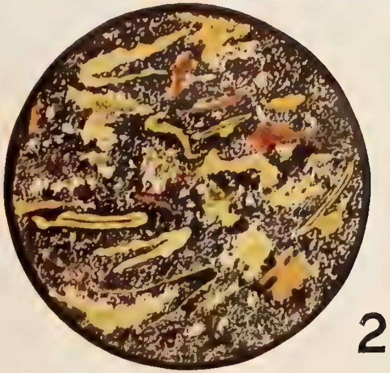

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PLATE 12.—The coloured Plate.

Figs. 1–4 are all on the same scale of magnification, and represent characteristic views of the four ingredients described in this paper, as seen in micro-sections of untreated coal, showing their natural colours as seen under the microscope.

Fig. 1.—Section of fusain. At the top, note portion of a bright coloured spore from the adjacent durain. The bulk of the section is still recognisable or macerated wood fibres, the walls of which are quite black and opaque.

Fig. 2.—Section of durain. Numerous crushed and partly broken spore coats, ranging from gold to orange colour, and various small fragments are interspersed with the black granular matrix.

Fig. 3.—Section of clarain. Tissues of a stem, spores and more or less translucent bodies of various kinds, are mingled.

Fig. 4.—Section of vitrain. Showing its essential structureless condition. The various shades of colour depending upon the thickness of the section, which is never quite uniform.

Fig. 5.—Tubes showing the approximate colour and the proportion of *débris* from the four ingredients after the treatment described on page 477. F, fusain, D, durain, C, clarain, V, vitrain.

The Comparative Rate of Absorption of Various Salts by Plant Tissue.

By WALTER STILES, M.A., Lecturer in Botany in the University of Leeds, and FRANKLIN KIDD, M.A., D.Sc., Fellow of St. John's College, Cambridge.

(Communicated by Prof. W. M. Bayliss, F.R.S. Received August 30, 1918.)

Introduction.

In a previous paper we have dealt with the intake by carrot and potato cells of single salts in different concentrations, and we there considered, especially, the dependence of the equilibrium attained in salt absorption on the concentration of the solution exterior to the tissue. In the course of these experiments dealing with the relation between concentration and intake, it was indicated that the rate of absorption and the position of equilibrium ultimately attained depended also upon the nature of the salt. As, however, experiments with different salts were made with different samples of tissue, we did not consider ourselves justified in laying much stress on the results obtained on account of the variability of different samples of tissue, a difficulty to which attention has previously been called. In the experiments recorded in this paper, we have therefore chosen one concentration and compared the conductivity changes produced in solutions of different salts by carrot and potato cells of the same sample.

Method.

The method used is that described in a former paper (12). The experiments were carried out in triplicate. All the results compared with one another were obtained with the same sample of tissue and from experiments carried on contemporaneously.

The concentration selected throughout was N/50, as with this concentration of salts of "nutrient" or harmless metals exosmosis is likely to be small in comparison with the absorption, so that the results will give a near approximation to the relative rates of absorption from the various solutions. When exosmosis takes place to any great extent the tissue loses water and its turgidity. Carrot in solutions of nutrient or harmless metals of a concentration of N/50 remained turgid right up to the conclusion of the experiments.

In each series the salts employed had a common anion or kation, so that the influence of different ions on the rate of absorption is displayed. Four series with carrot and three with potato were carried out, the common ions in the carrot series being respectively chloride, sulphate, nitrate, and potassium. Chloride, sulphate, and nitrate series were carried out with potato.

Experimental Results.

1. *Chlorides.*—The chlorides used were those of potassium, sodium, lithium, and calcium. The changes in conductivity in the case of carrot are given in the accompanying Table, and are shown graphically in fig. 1.

Table I.—Carrot in Solutions of various Chlorides of Concentration N/50.

Potassium chloride.		Sodium chloride.		Lithium chloride.		Calcium chloride.	
Time in hours.	Change in electrical conductivity.	Time in hours.	Change in electrical conductivity.	Time in hours.	Change in electrical conductivity.	Time in hours.	Change in electrical conductivity.
						0·25	−101
0·5	−145			0·5	−64	[illegible]·5	−113
1·0	−175	1·0	−80	1·0	−67	1·0	−113
		2·0	−117				
6·38	−335	6·62	−222	6·12	−119	6·20	−143
18·28	−758	18·62	−560	18·0	−257	18·0	−176
28·75	−1138	29·0	−896	28·5	−374	28·5	−230
43·25	−1497	43·5	−1228	43·0	−449	43·0	−341
49·25	−1595	49·5	−1329	49·0	−489	49·0	−371
91·25	−2039	91·5	−1677	91·0	−599	91·0	−628

From the Table and curves it will be observed that, in the first place, the apparent rate of absorption differs greatly with the different salts, and also that the initial rate does not depend on the position of equilibrium. There is little doubt that after 91 hours equilibrium is practically attained.

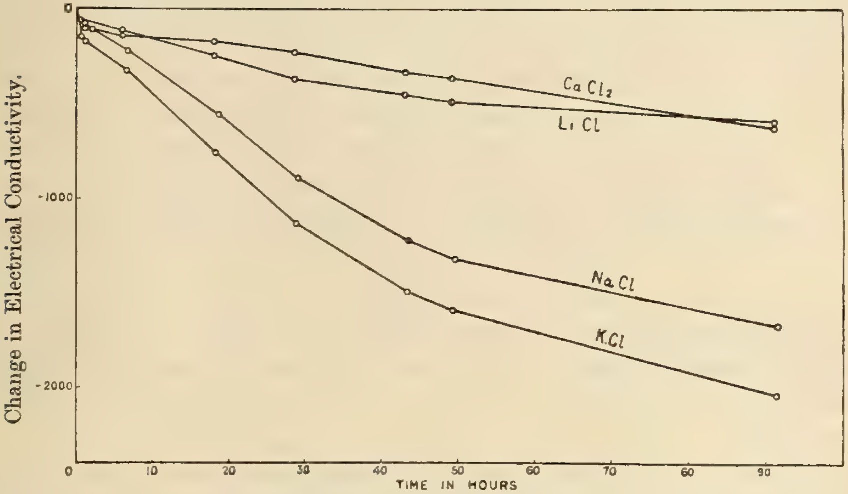

FIG. 1.—Carrot in Solutions of various Chlorides of Concentration N/50.

In N/50 solutions the four salts employed have almost identical ionic concentration, so that the difference in the rates of intake cannot be accounted for on the ground of differences in concentration. In Table II are given the degree of dissociation (which is proportional to the ionic concentration), and the mobility of the kation and the coefficients of diffusion in the case of the four salts. These physical constants are those given by Landolt and Börnstein (3).

Table II.—Absorption of Chlorides by Carrot. Mobility of Anion 65·4.

Salt.	Degree of dissociation.	Mobility of kation.	Coefficient of diffusion.	Decrease in conductivity after 1 hour.	Decrease in conductivity after 18 hours.
Lithium chloride ...	91	33·4	0·70	67	257
Sodium chloride ...	91	43·6	0·94	80	550
Calcium chloride ...	85	51·8	0·68	113	176
Potassium chloride	92	64·7	1·36	175	750

This Table suggests that the initial rate of salt absorption is dependent on the mobility of the kation. This result falls into line with the results obtained for hydrogen chloride (11). The mobility of the hydrogen ion is very high, namely 315, and it is found that the hydrogen ion is very rapidly absorbed from hydrogen chloride, and indeed from all acids (2).

The total amount of absorption is, however, as one would suppose, not conditioned by ionic mobility, but by some other factor, for calcium chloride, which after one hour has been absorbed to a greater extent than any other chloride except that of potassium, after 18 hours has fallen well behind all the others, which, however, maintain their relative positions. The initial order of absorption of kations from the chlorides thus appears to be K, Ca, Na, Li, while as regards total quantity absorbed it is K, Na, Li, Ca. The relative positions of lithium and calcium are a little doubtful, as at the end of the experiment there is actually a little more apparent absorption from calcium than from lithium chloride. It must be remembered that the numbers are only approximate, as they neglect exosmosis. Although this is undoubtedly negligible in the case of non-toxic solutions, yet lithium is generally regarded as somewhat toxic, and it is possible that there is more exosmosis from the tissue in the case of lithium than with the other chlorides (11).

In regard to the general course of absorption the curves are approximately logarithmic after the first few hours. During the initial period, however, the rate of withdrawal of salt from the solution is more rapid than it would be if the same logarithmic relation between time and intake held from the beginning. This suggests that there is an initial process at work rapidly withdrawing salt from the solution, which is followed by a slow general process which continues for several days before equilibrium is reached. If the results are expressed in terms of the percentage of salt required to produce equilibrium, the parts of the curves where the general process predominates all follow the same approximate course, showing that absorption proceeds to equilibrium at the same rate. Thus, although the initial rates of intake may be dependent on the mobility of the ions, in regard to the general course of absorption, the differences in rate of intake appear to be directly due to the position of equilibrium.

The initial absorption from solutions of the same salts by potato is similar, but the absorption is soon masked by the exosmosis that supervenes in the case of this more sensitive tissue. The numbers obtained experimentally are shown in Table III, and these results are exhibited graphically in fig. 2. The initial order of absorption is K, Na, Ca, Li.

Table III.—Potato in Solutions of various Chlorides of Concentration N/50.

Potassium chloride.		Sodium chloride.		Lithium chloride.		Calcium chloride.	
Time in hours.	Change in electrical conductivity.	Time in hours.	Change in electrical conductivity.	Time in hours.	Change in electrical conductivity.	Time in hours.	Change in electrical conductivity.
0·5	−116	0·5	− 87	0·5	− 33	0·5	− 70
3·0	−115	3·0	− 61	3·0	− 23	3·0	− 45
24·25	− 45	24·4	+ 66	24·5	+150	24·12	+136
45·25	+ 40	45·5	+190	45·5	+314	45·12	+219

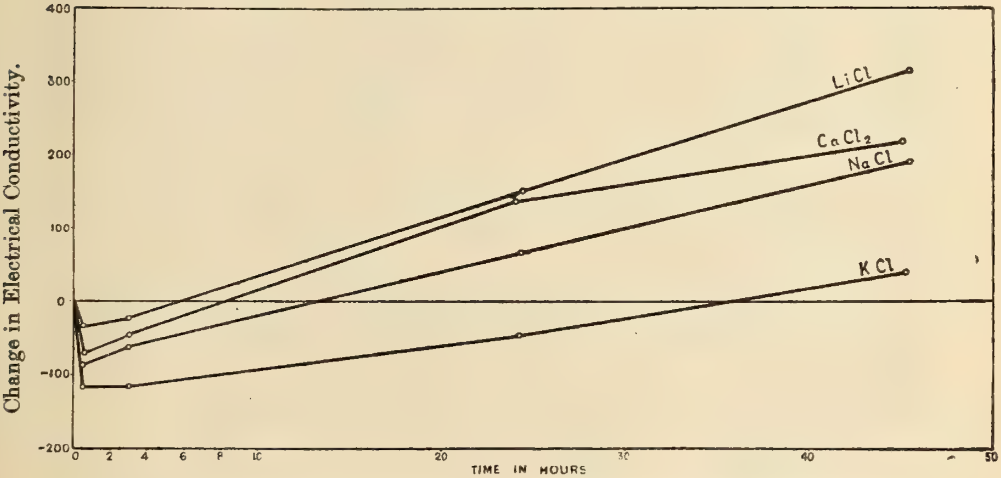

Fig. 2.—Potato in Solutions of various Chlorides of Concentration N/50.

2. *Sulphates.*—The salts employed in this series were the sulphates of potassium, sodium, magnesium, zinc, and aluminium. The first three are nutrient or harmless salts, zinc is generally regarded as a toxic element, while the anomalous behaviour of aluminium has been discussed in an earlier paper (12).

The results are such as might be expected. The greatest apparent absorption takes place with potassium sulphate, less with sodium sulphate, and still less with magnesium sulphate. With zinc and aluminium sulphates the electrical conductivity of the solution increases with continued immersion of carrot tissue in it. It will be observed that in the case of zinc sulphate a decrease in conductivity takes place during the first half hour corresponding to an absorption of the salt. Subsequently, the conductivity rises and continues to do so until the end of the experiment. This behaviour is completely explained on the basis of the absorption of the zinc; its toxic action brings about exosmosis which causes the subsequent rise in conductivity. The loss of turgidity of the discs corresponds with this.

Table IV.—Carrot in Solutions of various Sulphates of Concentration N/50.

Potassium sulphate.		Sodium sulphate.		Magnesium sulphate.		Zinc sulphate.		Aluminium sulphate.	
Time in hours.	Change in electrical conductivity.	Time in hours.	Change in electrical conductivity.	Time in hours.	Change in electrical conductivity.	Time in hours.	Change in electrical conductivity.	Time in hours.	Change in electrical conductivity.
0·5	−176	0·5	− 54	0·5	−37	0·25	− 13	0·25	+102
1·0	−183	1·0	− 48	1·0	−37	0·5	− 20	0·5	+139
14·25	−205	14·5	− 4	14·5	−26	14·0	+180	14·0	+381
25·25	−215	25·5	− 34	25·0	−26	25·0	+368	25·0	+451
43·0	−208	43·25	− 66	43·0	−12	43·0	+766	43·0	+644
64·5	−273	64·75	−117	64·5	−33	64·5	+929	64·5	+816

In the case of aluminium this explanation does not hold as the discs are perfectly healthy and turgid at the end of the experiment. We have suggested provisionally in a former paper that the aluminium ion is absorbed much more rapidly than the sulphate ion, and that the consequent replacement of the aluminium ion by one of a higher mobility, as, for instance, hydrogen, accounts for the increase of conductivity in this case. This point will be dealt with in more detail in a later section of this paper.

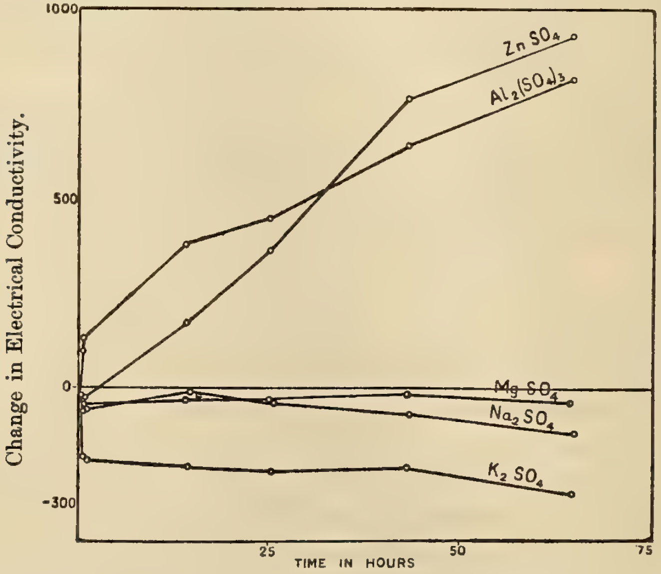

Fig. 3.—Carrot in Solutions of various Sulphates of Concentration N/50.

The initial rates of absorption indicated are in the order K, Na, Mg, Zn, and this order remains the same at the end of the experiment.

The figures given in Table IV, compared with those obtained for chlorides, show how much less is the apparent absorption from potassium and sodium sulphates than from the corresponding chlorides. The difference is so great that it can scarcely be referred to differences in the tissue used in the two series. Nevertheless, all the experiments in the sulphate series were repeated, and the same results obtained. A comparative series with anions given later confirmed this result.

The behaviour of potato in solutions of sulphates is very similar to its behaviour in solutions of chlorides. As before, the apparent initial absorption of the sodium salt is less than that of the potassium salt, but subsequent exosmosis prevents the obtaining of definite data as to the subsequent course of absorption by potato. The results obtained are shown in Table V, and graphically in fig. 4. They suggest the same order of absorption as with carrot. The order of the coefficients of diffusion of the four sulphates used is K, Na, Zn, Mg, while the order of absorption by carrot and potato is K, Na, Mg, Zn. There is thus a parallelism between the absorption and the coefficient of diffusion, although the position of zinc differs in the two series. It should be noted that the position of zinc in the absorption series is doubtful; as, on account of secondary changes resulting in exosmosis, more may be absorbed than appears from the observed results.

Table V.—Potato in Solutions of various Sulphates of Concentration N/50.

Potassium sulphate.		Sodium sulphate.		Magnesium sulphate.		Zinc sulphate.		Aluminium sulphate.	
Time in hours.	Change in electrical conductivity.	Time in hours.	Change in electrical conductivity.	Time in hours.	Change in electrical conductivity.	Time in hours.	Change in electrical conductivity.	Time in hours.	Change in electrical conductivity.
0·5	− 91	0·5	− 79	0·5	− 3	0·5	+ 15	0·5	+ 55
1·12	− 91	1·13	− 59						
14·0	− 25	14·0	+ 31	13·85	+101	14·3	+142	13·78	+200
20·0	+ 64	20·0	+101	19·87	+184	19·51	+170	19·28	+248
41·75	+225	41·75	+345	41·56	+336	41·28	+506	41·28	+643

3. *Nitrates.*—The nitrates of potassium, sodium, calcium, aluminium, and zinc were employed. The initial apparent absorption by carrot is very marked with potassium and sodium nitrates, less so with calcium and zinc nitrates, and slight but yet distinct with aluminium nitrate. With zinc

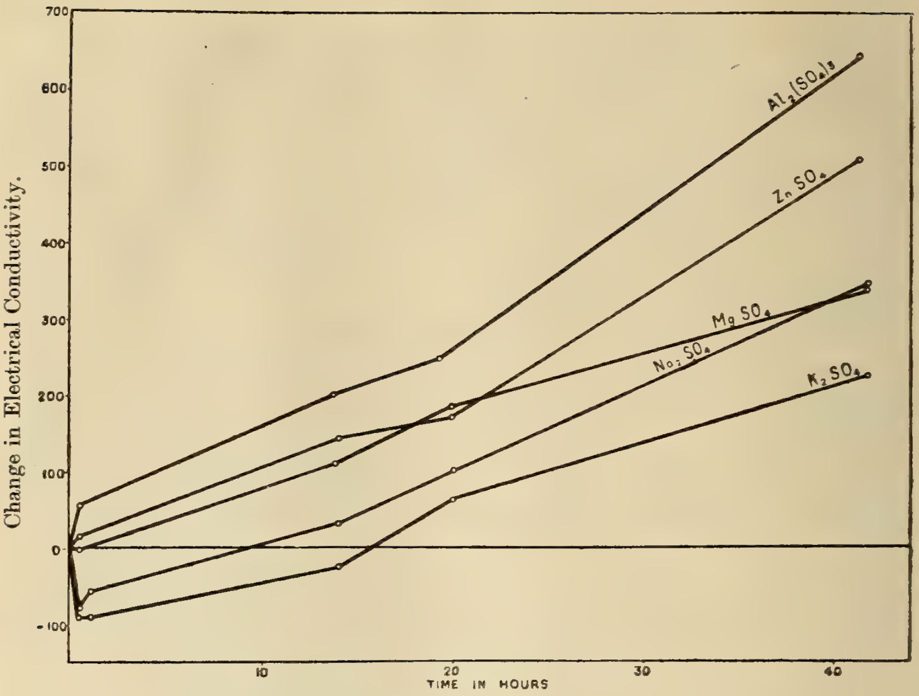

Fig. 4.—Potato in Solutions of various Sulphates of Concentration N/50.

nitrate the initial absorption is soon masked by exosmosis. The actual changes in conductivity observed are given in Table VI, and are shown graphically in fig. 5.

Table VI.—Carrot in Solutions of various Nitrates of Concentration N/50.

Time in hours.	Changes in conductivity of solutions.				
	Potassium nitrate.	Sodium nitrate.	Calcium nitrate.	Zinc nitrate.	Aluminium nitrate.
0·5	− 183	− 89	− 86	− 57	− 20
19·25	− 891	− 659	−215	+107	−135
71·50	−2023	−1360	−493	+540	−268

The relation of the initial absorption to the ionic mobilities and coefficients of diffusion is again suggested.

The subsequent decline in the rate of absorption of the calcium salt relative to the potassium and sodium is also in evidence. The toxic action of zinc is once more shown both by the subsequent rise in conductivity of the external solution and the loss of turgidity of the tissue. The tissue discs in the other solutions remained turgid at the end of the experiment.

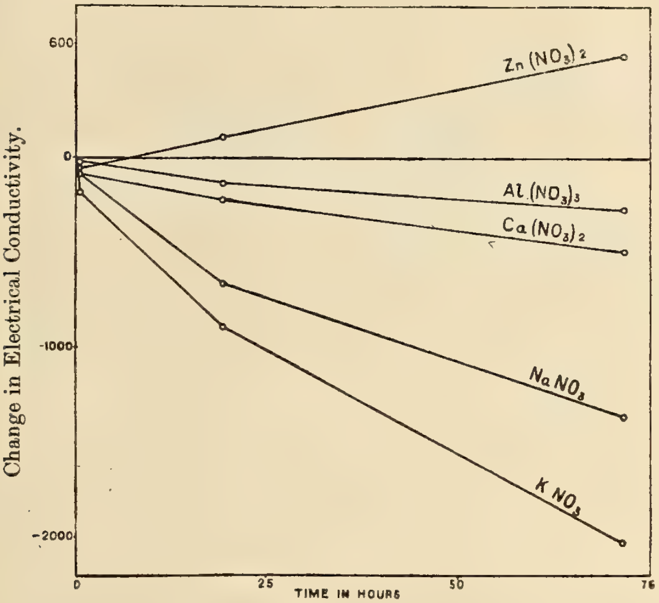

Fig. 5.—Carrot in Solutions of various Nitrates of Concentration N/50.

At the end of the experiment the reaction of the solution towards litmus was examined. The calcium and zinc nitrate solutions were neutral to litmus; whereas the potassium and sodium nitrate solutions were slightly acid, the aluminium nitrate was markedly acid.

The same series of nitrates were used with potato. As with other series, the same difference in the relation of absorption and exosmosis as compared with carrot is observable. The highest apparent exosmosis took place in the case of calcium nitrate. The results obtained are shown in Table VII and fig. 6.

The reactions of the solutions towards litmus at the end of the experiment were exactly the same as with carrot. The significance of this we shall discuss later.

4. *Potassium Salts.*—The results obtained with chlorides, sulphates, and nitrates indicate that the potassium ion is absorbed more rapidly than the other metallic ions used in these experiments. As, for the reasons we have given in a former paper, the values for absorption obtained by the conductivity method give approximately the intake of the less absorbed ion, in comparing the absorption of anions, we have chosen potassium salts, as the

Table VII.—Potato in Solutions of various Nitrates of Concentration N/50.

Potassium nitrate.		Sodium nitrate.		Calcium nitrate.		Zinc nitrate.		Aluminium nitrate.	
Time in hours.	Change in electrical conductivity.	Time in hours.	Change in electrical conductivity.	Time in hours.	Change in electrical conductivity.	Time in hours.	Change in electrical conductivity.	Time in hours.	Change in electrical conductivity.
0·75	−67	0·75	− 7	0·45	− 6	0·75	− 27	0·45	0
14·12	−95	13·97	+ 4	14·0	+ 60	14·10	+ 23	13·50	−141
20·70	−44			20·18	+127			19·35	−155
42·45	+67	41·77	+285	42·18	+382	41·93	+210	42·5	−181

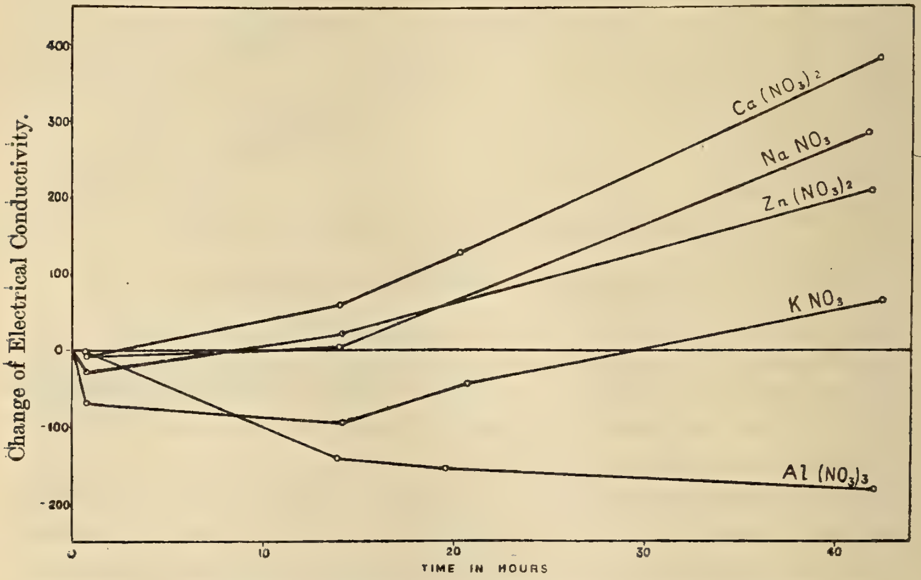

Fig. 6.—Potato in Solutions of various Nitrates of Concentration N/50.

measured decreases in conductivity should give approximate values for the intake of the anions.

The results obtained with carrot are shown in Table VIII and fig. 7.

These salts therefore produce the expected result. It will be observed that the original order of apparent absorption is potassium sulphate, potassium nitrate, and potassium chloride.

Nevertheless, the slowing down of the absorption from the sulphate solution due to the much smaller final absorption of sulphate (*cf.* the series with sulphates) later alters the order to potassium nitrate, potassium chloride, and potassium sulphate.

Table VIII.—Carrot in Solutions of various Potassium Salts in Concentration N/50.

Time in hours.	Change in electrical conductivity.		
	Potassium chloride.	Potassium sulphate.	Potassium nitrate.
0·25	− 145	−212	− 197
2·25	− 233	−258	− 214
19·0	− 550	−266	− 625
42·0	−1042	−311	−1152

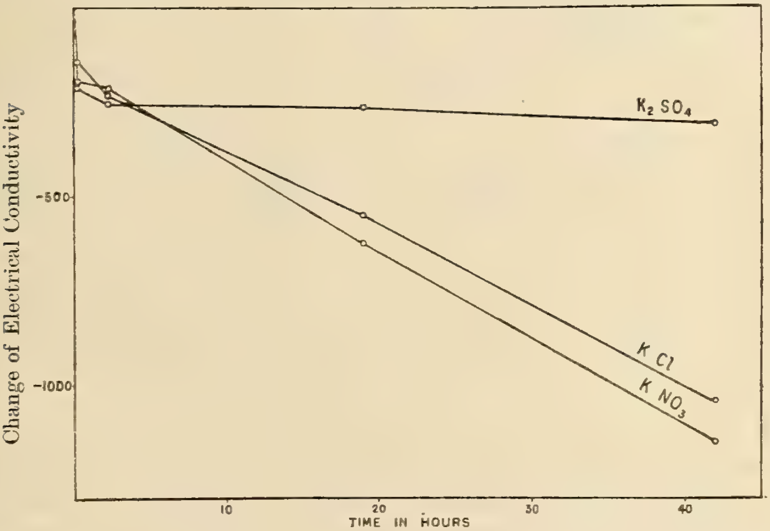

Fig. 7.—Carrot in Solutions of various Potassium Salts of Concentration N/50.

The difference between chloride and nitrate is small and no emphasis can be laid on it. The slight absorption of sulphate is very striking.

From these results it may be concluded that the order in which kations are absorbed at the beginning of the experiments is in general the following:—

K, [Ca, Na], Li, [Mg, Zn], Al,

although the nature of the anion may influence the order to some extent. The anions are absorbed initially in the order SO_4, NO_3, Cl. In both the case of kations and anions this order alters in the course of absorption, owing to the different position of equilibrium attained in the cases of the various salts. The following Table shows the influence of the nature of the salt on the value of the absorption ratio (*i.e.*, the ratio between internal to external concentration of the salt or ion) after the absorption has proceeded towards equilibrium.

as far as the conditions of experiment allow. The most noteworthy fact brought out from this Table is the much smaller total absorption of calcium and magnesium than of potassium and sodium, and the much smaller total absorption of sulphate than of chloride and nitrate. Potassium is always absorbed to a slightly greater extent than sodium, while the indication is that nitrate is absorbed somewhat more than chloride. The results will now be discussed in the following section, where the results of some other workers will be compared with those here recorded.

Table IX.—Ratio of Salt Concentration in Tissue to that in External Solution after Intake of Salt from Solutions in N/50 Concentration having a common Kation or Anion.

Salt, N/50.	Absorption ratio.	Salt, N/50.	Absorption ratio.
Chloride series, 91 hours—		Nitrate series, 71·5 hours—	
KCl	3·58	KNO_3	4·65
$NaCl$	3·49	$NaNO_3$	3·30
$LiCl$	1·16	$Ca(NO_3)_2$	1·19
$CaCl_2$	1·09	$Al(NO_3)_3$	0·53
Sulphate series, 64·5 hours—		Potassium series, 42 hours—	
K_2SO_4	0·51	KCl	1·99
Na_2SO_4	0·46	K_2SO_4	0·55
$MgSO_4$	0·097	KNO_3	2·20

Discussion.

In an earlier paper we have shown that the rate of intake of salts by plant tissue depends on the concentration of the solution from which absorption takes place, and that the equilibrium reached in salt intake depends very greatly on the concentration, a relatively less proportion of the salt being absorbed with increasing concentration. In attempting to determine the relative rates of absorption of different salts or ions it is therefore essential that the salts compared should be presented to the tissue in equivalent concentration. The data recorded in these pages have been obtained with concentrations of N/50.

It is first necessary to discuss how far the results obtained by the conductivity method justify a comparison of the rates of intake of different salts.

If the salt is absorbed without exosmosis resulting, and if the two ions of the salt are absorbed in equivalent quantities, the decreases in conductivity will be very close measures of the absorption of the salt. The increase in the degree of dissociation resulting from dilution will be slight, and as all the salts compared are absorbed more or less, a slight increase in the degree of dissociation will result from this in all the solutions, which reduces any slight error arising from changes in the degree of dissociation resulting from dilution. Moreover, the greatest increase in the degree of dissociation is to be expected in the solutions from which most salt is absorbed. Hence, with greater absorption, the decrease in conductivity tends to give a value for the absorption less than the true value, from which it follows that changes in the degree of ionisation consequent on absorption of salt and the resulting dilution of the solution will not affect the comparison of the rates of absorption of different salts, except to make the differences appear slightly less than they actually are.

A more serious complication arises from exosmosis. We have formerly given reasons why, in the case of carrot immersed in solutions of nutrient and harmless substances, this source of error is not of importance, whereas with potato it makes the drawing of conclusions much more difficult. Hence, our conclusions are based primarily on the results obtained with carrot; the data from experiments on potato are subsidiary to and confirmatory of those from experiments on carrot. Among the results with the latter the toxic action of zinc shows clearly the influence of exosmosis in obscuring the absorption.

A third difficulty arises if the two ions of a salt are not absorbed equally. Nathansohn (5, 6), Meurer (4), Ruhland (10), and Pantanelli (7) have shown by direct chemical analysis that the ions of a salt are not necessarily absorbed with equal rapidity. In the case of inequality of ionic absorption the excess of ion absorbed must be replaced by another ion of the same sign, which may be brought about either by exosmosis from the tissue or by hydrogen or hydroxyl ions. If the absorption of the two ions differs greatly, and if the replacing ion differs markedly in mobility from that of the ion absorbed in excess, the fall in conductivity will no longer give a measure of the absorption.

Among the experiments recorded in this paper, those with aluminium are probably to be explained on these grounds. It will be recalled that with aluminium sulphate, although the discs remain turgid and show no sign of injury, yet the conductivity of the solution rises, thus simulating the state of affairs in a toxic solution. Now, Rothert (8) and especially Meurer (4) have shown by direct analysis that the aluminium ion is absorbed to a very great extent from solutions of aluminium sulphate. The results of Pantanelli (7) with a variety of plants are equally striking. We have collected Pantanelli's results with regard to absorption of aluminium salts in the following Table, which show how widely distributed throughout the plant kingdom is the rapid absorption of the aluminium ion in comparison with the sulphate ion :—

Table X.—Absorption of Aluminium (Data from Pantanelli).

Plant organ.	Salt.	Concentration.	Ratio of equivalents of $\frac{\text{kation}}{\text{anion}}$ absorbed.
Vicia Faba root	$Al(NO_3)_3$	0·0125 M	1·008
Cicer arietinum root	$Al(NO_3)_3$	0·0125 M	1·570
,, ,,	$Al_2(SO_4)_3$	0·0125 M	2·533
Azolla	$Al_2(SO_4)_3$	0·0125 M	8·513
Barbera wine yeast	$Al_2(SO_4)_3$	0·05 M	3·681

As a consequence of this great inequality of absorption, we should expect either an exosmosis of a kation from the tissue, or the replacement of the aluminium ion by hydrogen ion from the solvent, the corresponding quantity of hydroxyl ion being absorbed with the aluminium. In the latter case the solution would become acid, and this is indeed the case, as we have ourselves observed. As the mobility of the hydrogen ion is so much greater than that of other kations, the increase of conductivity of the external solution without exosmosis into it is at once explained.

The results of Pantanelli summarised above also show that with aluminium nitrate the absorption of the aluminium and nitrate is more equal. This agrees with the results obtained by us, for with aluminium nitrate there is no rise in conductivity of the external solution, but a progressive decrease as in the case of salts of the alkali metals.

With those harmless salts which show in our experiments with carrot a progressive decrease in conductivity, it seems reasonable to conclude, therefore, that the excess of absorption of one ion over the other is much less than in the case of aluminium nitrate. Moreover, in each series all the salts employed had a common ion, and it is reasonable to suppose, therefore, that in any one series the order in which the differing ions are absorbed is given by the order in which the conductivities of the external solutions are lowered.

We have shown in the part of this paper dealing with the results of experiments that the initial order of intake of the ions is not necessarily the same as the final order. This, for instance, is especially noteworthy in the case of the sulphate ion, which appears to be absorbed more rapidly in the beginning than the nitrate or chloride ion, but of which the total quantity absorbed is considerably less than in the case of these monovalent anions.

The *initial* order of absorption of kations as indicated by our experiments is as follows: K, [Ca, Na], Li, Mg, Zn, Al; it is possible the positions of Ca and Na should be reversed. The *final* order is, however, the following: K, Na, Li, [Ca, Mg], the chief difference being in the position of calcium

The anions are absorbed initially in the order SO_4, NO_3, Cl, but this gives place later to the order NO_3, Cl, SO_4, owing to the slowing down of the rate of absorption of sulphate.

These conclusions agree well with the results of earlier workers. Ruhland (9) showed that potassium and sodium nitrates penetrate the protoplasm of *Spirogyra* more rapidly than calcium nitrate, and potassium and sodium chlorides more rapidly than calcium chloride. Potassium and sodium nitrates penetrate more rapidly than the corresponding chlorides. The (final) order of kation absorption by *Spirogyra*, according to Ruhland, is therefore [K, Na], Ca, and the order of anions: NO_3, Cl.

Fitting (1) has recently followed the rate of intake of salts by the epidermal cells of *Rhoeo discolor* by following the rate of deplasmolysis in hypertonic solutions. He records that potassium nitrate and chloride penetrate the protoplasm with ease, but the rate of penetration of potassium sulphate is much smaller. As regards kations, sodium nitrate and sodium chloride both penetrate the protoplasm, but lithium chloride and lithium nitrate are less permeable than potassium nitrate. Magnesium chloride, nitrate and sulphate are only permeable in small degree, while in regard to calcium chloride and calcium nitrate Fitting could observe no permeability. The same applies to barium salts.

The order found by Fitting is thus as follows: for kations [K, Na], Li, Mg, [Ca, Ba]; for anions [NO_3, Cl], SO_4.

Troendle (13) using the same method as Fitting on roots of *Lupinus albus* and palisade cells of the leaves of *Acer platanoides* and *Salix babylonica*, finds varions ions absorbed in the following order: kations, Rb, K, Na, Li, Mg, Ba, Sr, Ca; anions, NO_3, Cl, SO_4.

Pantanelli's researches, to which reference has been made earlier, extend over a wide range of forms, including flowering plants (e.g., *Cicer arietinum*, *Vicia Faba*), green algae (*Valonia utricularis*, *Ulva lactuca*), and a yeast (Barbera Wine yeast). His results show that a considerable variation occurs in different forms. With higher plants, the absorption of calcium is markedly less than that of potassium, while the rapidity of the absorption of nitrate is clearly shown. With lower plants, on the other hand, calcium is readily absorbed. Pantanelli's results bring out clearly the different rates of absorption of the kation and anion of the same salt, but it is also clear from comparison of the absorption of the same ion from different salts, that the absorption of one ion is influenced by the other. For example, *Cicer arietinum* absorbed 0·35 mgrm. of potassium from a 0·025 N solution of potassium chloride, while from a solution of potassium nitrate of the same concentration 2·74 mgrm. of potassium were absorbed in the same time.

In general, therefore, it may be concluded that earlier work agrees with our own as to the relative degree of absorption of different ions. The remarkable thing is that the differences between different tissues in this respect are as small as they appear to be.

In general it would appear that the initial rate of absorption of kations is influenced very largely by the mobility of the ion or the coefficient of diffusion of the salt. Troendle, on the contrary, draws the conclusion that the rapidity of entrance of ions is not controlled by the force of diffusion of ions and molecules through the protoplasm into the vacuole. He draws this conclusion as a corollary to an earlier one which he reached, as we think (12) on insufficient grounds, that the rapidity of entrance of ions is independent of the concentration of the salt. We have already pointed out how our own results as to the influence of concentration contradict the conclusion of Troendle as to the influence of concentration, although we agree with him that the absorption of salt is not governed by Fick's law of diffusion. Troendle concludes that the rapidity of penetration of salts depends on the nature of the kation, and that the rapidity of entrance increases with the atomic weight of the kation in any one group of the periodic classification. This certainly appears to be the case with the alkali metals, but Troendle admits that magnesium contradicts his rule, as he found it was absorbed more rapidly than calcium. Our own numbers suggest that calcium is absorbed more rapidly than magnesium at first, a fact which would support Troendle's opinion, but how far this is a general phenomenon remains to be seen.

Troendle and earlier workers have not differentiated between the initial rate of absorption and the position of equilibrium which is finally attained. The results recorded in this paper show clearly that the initial order of absorption is not maintained. Thus, calcium and sulphate, which are initially absorbed at a rate equal to or greater than that of sodium and chloride respectively, are absorbed ultimately to only a third or a quarter of the extent to which these monovalent ions are taken in. How far this is a distinguishing feature of divalent as contrasted with monovalent ions cannot be said, although magnesium appears to behave like calcium. We know, however, that the trivalent aluminium ion is absorbed to a large extent, but this may be to a considerable extent, as Meurer supposes, by the cell wall and not by the cell interior, and so may be an exceptional case.

We would conclude, tentatively, that the initial rate of absorption of salts is dependent to a great extent on the mobility of the ions or the coefficient of diffusion of the salt, but that the total intake of salt depends on something other than this, as a result of which calcium, magnesium, and sulphate are absorbed to a much less extent than potassium, sodium, chloride, and nitrate.

Summary.

1. The rate of absorption of various chlorides, sulphates, nitrates, and potassium salts from solutions of 0·02 N concentration was measured by the electrical conductivity method previously described.

2. After a brief initial period, lasting only a few hours, of rapid withdrawal of salt from solution, there follows a long period, lasting several days, during which absorption proceeds to an equilibrium. Over this period the curve follows an approximately logarithmic course.

3. Kations appear to be absorbed initially in the following order: K, [Ca, Na], Li, [Mg, Zn], Al. The position of ions enclosed within brackets may have to be reversed. This initial order of the rate of absorption does not indicate, however, the extent to which the ions are absorbed when equilibrium is approached; the order of absorption is then K, Na, Li, [Ca, Mg], the chief difference between this order and the initial order being in the position of Ca, which is absorbed to only a slight extent compared with K and Na.

4. Anions appear to be absorbed initially in the order SO_4, NO_3, Cl, which gives place later to the order NO_3, Cl, SO_4 on account of the comparatively slight extent to which the sulphate ion is absorbed. The difference between nitrate and chloride is slight, and stress should not be laid on it.

5. These results agree in general with those of Ruhland, Fitting, Pantanelli, and Troendle, using different methods and different experimental material. These workers, however, did not distinguish between differences in the initial rates of absorption and differences in the position of equilibrium.

6. It seems clear that the rate and extent of intake of one ion of a salt may be influenced by the nature of the other ion.

7. The results obtained in regard to aluminium support the observations of Rothert and Meurer that this ion is rapidly absorbed from aluminium sulphate. This proceeds much more rapidly than the absorption of the anion.

8. Although Troendle's view that in any group of the periodic classification the metallic ions are absorbed more rapidly the higher the atomic weight, is not contradicted, yet the view that the initial rate of absorption is largely dependent upon the mobility of the ions or diffusibility of the salt is equally well supported, and can be put forward provisionally as a more reasonable hypothesis.

The position of equilibrium appears, however, to be governed by some quite different property, as to the nature of which it would be premature at present to make a suggestion. Our results are that the divalent ions, Ca, Mg, and SO_4, are at the final equilibrium absorbed to a much less extent than the monovalent ions, K, Na, Cl, and NO_3.

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Changes in the Teeth of the Guinea-pig, produced by a Scorbutic Diet.

By S. S. Zilva, Ph.D., M.Sc., and F. M. Wells, Major, C.A.D.C.

(Communicated by Dr. A. Harden, F.R.S. Received December 6, 1918.)

(From the Biochemical Department, Lister Institute, and the C.A.D.C. Laboratory.)

[Plate 13.]

The pathological anatomy of adult and infantile scurvy has received the attention of many investigators at various times, and is now known in great detail. Not only have the macroscopic but also the microscopic changes been fully studied. Definite characteristic abnormalities in the tissues of the bones, and at the costochondral junctions and the epiphyseal lines in scorbutic subjects, are well recognised. Although the gums are known to be greatly affected in advanced cases of scurvy and the teeth become loose, there has apparently been no chemical or histological work done hitherto on the teeth, since no references to such work can be found in the literature. This is most probably due to the fact that most of the histological work has been carried out in infantile scurvy.

During the last few years, research in scurvy has been greatly stimulated by the discovery of the possibility of inducing experimental scurvy in animals. Holst and Frölich (1912) have shown that a pathological condition analogous to scurvy in the human subject can be induced in guinea-pigs by dietetic means. That the condition of the animals thus produced was really scorbutic was further confirmed by the observations of these investigators that it could be prevented by the addition of antiscorbutics to the deficient diet. These observations have since been fully corroborated by many workers. Hart and Lessing (1913) have further demonstrated that monkeys when fed on a diet deficient in antiscorbutic develop scurvy. This has been confirmed by Talbot, Dodd and Peterson (1913), and by Harden and Zilva (1919). The latter investigators have also shown that a monkey suffering from scurvy could be cured by administering an antiscorbutic.

The present communication describes the changes observed in the structure of the teeth of scorbutic guinea-pigs. One of us (S. S. Z.), being engaged in an extensive investigation of experimental scurvy, was in a position to examine many guinea-pigs and monkeys in a scorbutic condition. It soon became apparent from the macroscopic appearance that the teeth underwent a great change. Accordingly, Major Wells was consulted on the dental aspect of the subject, and, as the result of his preliminary examination of some of the teeth, it was decided to investigate the matter jointly in detail.

We had the opportunity of examining a great number of teeth derived from guinea-pigs in various stages of scurvy. Some of the animals succumbed to scurvy on an unsupplemented scorbutic diet, others received, in addition to the scorbutic diet, solutions of weak antiscorbutic potency, but active enough to delay the onset and the fatal termination of the disease; others, again, received doses of various preparations which turned out to be quite inactive. The teeth of normal guinea-pigs were also examined as controls.

We have convinced ourselves that the mildest degree of scurvy which could just be discovered at the *post-mortem* examination produced well-defined changes in the structure of the teeth, and in our numerous examinations we have not observed a single exception to this statement.

In our earlier observations the teeth were ground and the hard unstained sections were examined. This mode of examination revealed most of the features we are about to describe, but we thought it desirable, in order to bring out all the details more plainly, to adopt a convenient method of staining soft sections of the teeth. Such a method was suggested to us by Dr. P. P. Laidlaw, of Guy's Hospital, and we take this opportunity of expressing our gratitude to him for the pains he took in helping and advising us. Without his help and advice this investigation would have lacked many important details.

Method of Investigation.—For the purpose of this enquiry the lower incisor and molar teeth of the guinea-pig were chosen. As in all rodents, these teeth grow from persistent pulp and are never shed. The teeth while still *in situ* in the lower jaw were decalcified and sections made in an antero-posterior direction, parallel to the long axis. The teeth were decalcified in a solution containing 35 per cent. of formalin, 42 per cent. of formic acid, and 23 per cent. of distilled water. When decalcification was complete, sections were made by freezing the material in gum and then staining with hæmatoxylin. Some specimens were decalcified with phloroglucinol and nitric acid, which possesses the advantage of bringing about the decalcification much more quickly.

In advanced cases of scurvy the teeth were apparently sound, but useless, inasmuch as they had been loosened by the gradual absorption of the cement membrane of the alveolar sockets, which had left exposed that portion below the neck. As a result there must have occurred that periostitic pain or something analogous which follows in the case of human patients who are suffering from shrunken alveoli. These teeth also presented, in addition, all the appearances of the changes of senility. A great number of longitudinal and transverse sections suitable for microscopic examination were obtained. The revelations offered by these sections are of a particularly interesting nature. It would require too much space to describe all the sections of teeth examined and we, therefore, consider it more appropriate to sum up the changes that take place.

Figs. 1 and 2 (Plate 13) are of a normal tooth and give one the opportunity of studying the histology of the dental pulp in its normal relationship to dentine. The enamel is not quite so heavy as in the natural tooth, on account of the decalcifying process which has reduced it to about half its thickness, but it gives one an excellent example of the typical relationship to the dentine of the blood vessels, the fine cellular tissue, and odontoblastic cells when in a normal condition. Note the fine-cut lines of the dentine and odontoblastic cells as compared with figs. 3 and 4.

Figs. 3 and 4.—It is obvious that the term "fibrosis" or fibroid degeneration, is the only one which can with certainty be applied to this particular form. There is no doubt that it is a specimen of degeneration, and it is equally easy to eliminate those other degenerative varieties, such as mucous, calcareous, or fatty, which animal tissues may undergo.

The present instance affords an opportunity of examining certain structural metamorphoses in the pulp, which are believed not to be dependent on any inflammatory condition, but simply attendant on and produced by altered metabolism or constitutional changes due to diet. This affection seems to have been unknown or overlooked by the pathologists both in Europe and America. Minute descriptions have been published on pulp nodules, calcareous pulps, and elaborate work on ulcers and tumours connected therewith. In no case does one find the condition as depicted in figs. 3 and 4 brought about by a dietetic experiment.

It is evident in this picture that in complete pulpar fibrosis no cellular elements of any description occur. It is clear at once and it is an important fact that no trace of cellular organisation, no trace of cell nuclei, no trace of interstitial cement substances can be found anywhere. Nerves, cells, blood vessels and odontoblasts have all shared the process of fibrification and are no longer recognisable. The fine cellular connective tissue which is but a loose mass of network in the normal state, has either become grossly hypertrophied or quite obliterated, and its place taken by a new firm, fibrous structure, devoid of cells, nuclei, or any regular arrangement of constituted parts. Figs. 3 and 4 show an advanced state of scurvy. The irregular osteoid condition of the dentine is well marked and the different refractive appearance of the dentine is probably due to the hæmorrhagic condition of the dentinal fibrils.

In a scurvy tooth the condition persists right up to the apex of the root; the change appears to start first in the odontoblastic cells at the top of the pulp working down towards the apex, followed by distended blood vessels and hæmorrhage; then complete fibroid degeneration follows.

These sections are typical of the great number of teeth examined.

It has already been pointed out that the changes in the structure of the teeth is a feature observed in quite early stages of the disease. An experiment was devised to demonstrate this systematically. Four sets of two guinea-pigs each were put on a scorbutic diet of oats, bran, and autoclaved milk. In the ordinary course, animals subsisting on such a diet cease to grow after about 15 days and then commence declining in weight and eventually succumb to scurvy within a period varying from 21 to 28 days after the commencement of the experiment. After about the 15th day such scorbutic symptoms as tenderness of the limbs can be observed. Animals chloroformed at this stage usually show, at the *post-mortem* examination, intramuscular hæmorrhages, especially in the femoral muscles, subcutaneous and subperiosteal hæmorrhages, enlarged costochondral junction and fragility of bone which is well marked near the epiphyseal line in the tibia and the femur. The entire pathological picture is characteristic of scurvy. In the experiment the following procedure was adopted. The groups of animals were chloroformed after they had subsisted on the scorbutic diet for 7, 10, 12, and 17 days respectively. A careful *post-mortem* examination was carried out on the animals, and stained sections of the teeth of the respective groups were examined microscopically.

The results are summarised in the following Table. The degree of change in the structures of the teeth is indicated by the number of crosses in the Table. Two outstanding features are brought out by this experiment. In the first place it becomes plain that the tooth is one of the first, if not the first part of the system to be affected by the deficiency of antiscorbutic material in the diet. The second point to be marked is that when the scorbutic symptoms during life are so slight as to be almost unrecognisable, profound changes in the tooth are recorded. If one considers the animals No. 366, 368, 369 (367 showed the idiosyncrasy of being rather resistant to scurvy), one sees that these animals were in apparent good health and gaining in weight. The scorbutic changes disclosed by the *post-mortem* examination were such as could hardly have produced any discomfort to the animals, yet the change in the teeth was profound. We shall refer to this later.

All the guinea-pigs so far examined were several weeks old, and experiments were therefore instituted with the object of seeing whether similar changes could be observed in animals put on scorbutic diet soon after birth.

Table.

No.	Duration of experiment.	Initial weight.	Final weight.	*Postmortem* remarks.	Condition of teeth.
	days.	grm.	grm.		
372	7	362	372	Normal	Normal.
373	7	385	384	Normal	Blood vessels slightly dilated.
366	10	254	323	Slight hæmorrhages in femoral muscles and along lower ribs. Everything else normal.	+ +
367	10	285	370	Normal	Normal.
368	12	258	327	Slight hæmorrhages in femoral muscles and along lower ribs. Slight "beading" at costochondral junctions.	+ +
369	12	246	306	Slight hæmorrhages in femoral muscles; bones somewhat brittle. Slight "beading" of costochondral junctions.	+
370	17	241	270	Very marked hæmorrhages in femoral muscles. Enlarged and hæmorrhagic costochondral junctions. Shafts of femur and tibia brittle near epiphyseal junctions.	+
371	17	265	254	(Same as 370)	+ + +

This was possible with guinea-pigs, as these animals can maintain themselves when removed from their mother immediately after birth. The following protocols describe three representative cases:—

1. Guinea-pig No. 316, born on May 30, 1918, was put immediately on a diet of oats, bran, and autoclaved milk. It weighed 89 gram. at the time of its birth. On June 14, 1918, it reached its maximum weight of 135 gram. From that day it commenced losing in weight and died on June 24, 1918, weighing 113 gram. At the *post-mortem* examination it was found that the intestinal walls appeared to be hæmorrhagic, although no hæmorrhages were found elsewhere. The bones of the fore and hind limbs were very soft. There was decided "beading" at the costochondral junctions. The ribs of this and the following animals were kindly examined for us, histologically, by Miss F. M. Tozer, who found fractures and disorganised junctions of the cartilage and bone. The structure of the teeth of this animal was modified to a marked extent and was assessed by us to be + +.

Guinea-pig No. 313, born May 30, 1918, weighing 97 gram., was put on scorbutic diet of oats, bran, and autoclaved milk immediately after birth. It received also occasional small doses of orange juice, enough to delay the fatal termination but not adequate to prevent scurvy. After about 15 days the animal displayed definite systems of scurvy. It was kept in this condition until July 23, 1918, when it died, weighing only 128 gram., which was far below the average weight (about 200 gram.) of a normal guinea-pig of that age. The *post-mortem* examination revealed very brittle bones, old hæmorrhages in the femoral muscles, and at the costochondral junctions no definite beading but merely a narrow ridge. The histological examination of the costochondral junctions showed nothing abnormal. The changes in the teeth were profound, and were assessed by us at the maximum indicated by + + +.

Guinea-pig No. 273 was born April 18, 1918, weighing 62 gram., and was put on scorbutic diet of oats, bran, and autoclaved milk the same day. Like the preceding animal it received occasional small doses of orange juice. The animal, after about a fortnight, manifested symptoms of scurvy, and was kept in a chronic state of the disease for two months until June 18, 1918, when it weighed 97 gram. It was then put on a normal mixed diet. Several days after (June 29, 1918), owing to the very brittle condition of the teeth, a part of the lower incisor broke off. This was examined by us histologically, and was found to be in a highly deteriorated condition, as shown by photograph (5). After being put on a mixed diet the animal commenced gaining in weight. As its teeth continued growing one could definitely distinguish two zones, coinciding with the two periods of nutrition. The lower zone was white and quite normal, the upper was yellow, with less perfect enamel. This guinea-pig was chloroformed July 19, 1918. At the *post-mortem* examination nothing abnormal was found. The teeth still manifested some change in structure, assessed by us as +.

These experiments show that these very young guinea-pigs behaved in precisely the same way as the older animals.

The radical changes in the teeth, brought about by the deficiency of the diet in antiscorbutics, has also been definitely established in the monkey. This investigation is, however, as yet incomplete, and the results are therefore deferred for a future communication.

It is necessary at this juncture to point out the identity of guinea-pig, monkey, and human scurvy. It would be useless repetition to indulge in a complete discussion of the pathological anatomy. This has already been ably done by Holst and Frölich (1912) with the guinea-pig, and Hart and Lessing with the monkey. Recent discoveries throw, however, a very interesting light on the subject. Harden and Zilva (1918) have shown that by removing the organic acids from lemon juice an antiscorbutically potent residue remains behind, which is almost equal in activity to the original juice. This was ascertained by them protectively on guinea-pigs and curatively on a monkey. The preparation has recently been tried by Harden, Zilva, and Still (1919) on several cases of infantile scurvy, with results that have fully corroborated the observations made on animals. This fact establishes beyond doubt the identity of the disease in the three cases.

In drawing from the above experiments conclusions bearing on human subjects, one is fully alive to the fact that more work, both of an experimental and statistical nature, will have to be done before definite conclusions can be drawn. At present, no satisfactory explanation has been advanced for the great prevalence of tooth decay amongst civilised communities. This investigation suggests that deficiency in diet forms a reasonable working hypothesis on which future research may be based. It is true that adult and even infantile scurvy is more or less rare in most civilised countries, but this applies to the well declared form of the disease. Our animal experiments show definitely that the scurvy may be of an extremely mild form, and yet produce very marked changes in the teeth. Hess (1917) shows how children may suffer from "subacute" scurvy by no means easily recognised. He further defines another type, which he calls "latent scurvy," which is a state of malnutrition, and can only be diagnosed by the improvement in the state after the administration of an antiscorbutic. It is evident that such transient conditions of infantile scurvy may occur more often than is usually suspected, and may reasonably be expected to influence dentition. It has already been pointed out that it is not advisable to speculate too much at this stage. One thing is certain, that the problem invites further investigation. Our knowledge of nutrition has advanced rapidly during the last decade. Besides the antiscorbutic factor there are at least two other accessory factors which are vital to our existence. One is the antineuritic, sometimes called the anti-beri-beri "vitamine," and the other the "fat soluble" factor discovered by McCollum and Davis (1914). That the polyneuritic condition known as beri-beri is produced by the dietetic deficiency of the former is now almost universally acknowledged. The actual pathological changes in human subjects caused by the dietetic deficiency of the "fat soluble" factor have not yet been defined, but that it is indispensable for rats has now been shown beyond doubt. Other nutritional irregularities due to protein deficient in certain amino-acids, or to certain inorganic constituents of the diet, are also of supreme importance, and it would be of interest to study their effect on the teeth.

An investigation, in which some of these factors are being studied for a different purpose, is at present being carried on at the Lister Institute, and it is hoped that the *post-mortem* material may be utilised for further dental investigations, with the object of throwing more light on the function of the diet in dental hygiene.

S. S. Z. is responsible for the biochemical part of the investigation; F. M. W. for the histological work.

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DESCRIPTION OF PLATE.

Fig. 1.—Longitudinal section through normal guinea-pig tooth. The pulp is *in situ*. Prepared by decalcification. Stained with Ehrlich's acid hæmatoxylin. Magnified 40 times. D, dentine; B, blood-vessels; O, odontoblasts; E, enamel; B.L., basal layer of Weil.

Fig. 2.—Same as preceding figure. Magnified 200 times. O, odontoblasts; P, pulp tissue; B, blood-vessels.

Fig. 3.—A transverse section of a guinea-pig tooth kept on a scorbutic diet for about 15 days. D, dentine; E, enamel; P, dead pulp, showing complete degeneration of the pulp. Magnified 40 times, stained as in preceding figure.

Fig. 4.—Same as fig. 3. Magnified 200 times. Prepared and stained as in preceding figure. D, dentine; D.O., degenerate odontoblasts; P, dead pulp tissue proper.

Fig. 5.—Longitudinal section through piece of tooth of guinea-pig No. 273, broken off 29.6.18. P, pulp; D, dentine. Stained and prepared as in preceding figures. Magnified 40 times.

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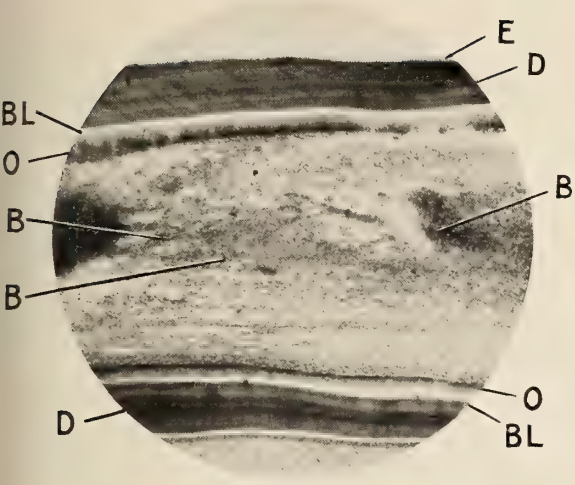
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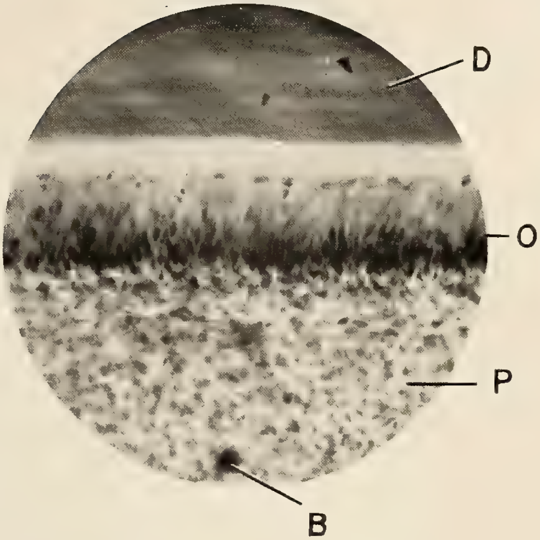

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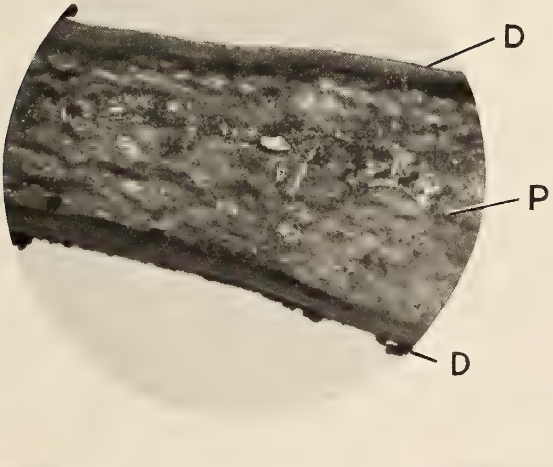

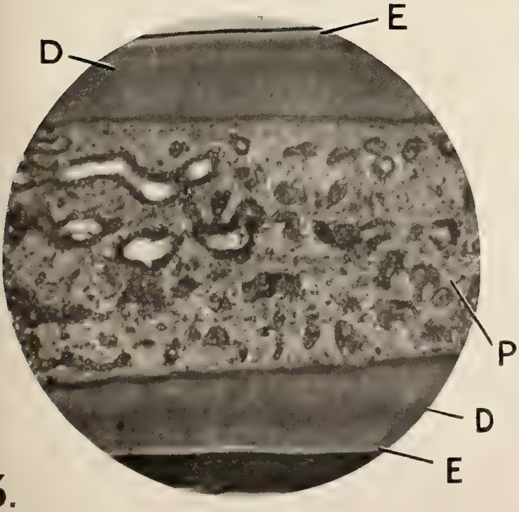

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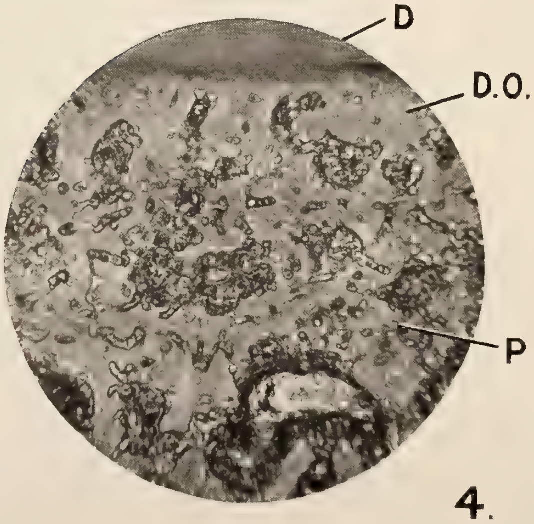

4.

On a New Factor in the Mechanism of Bacterial Infection.

By W. E. Bullock and W. Cramer.

(Communicated by Prof. W. Bulloch, F.R.S. Received January 14, 1919.)

(From the Laboratories of the Imperial Cancer Research Fund.)

Introduction.

The observations recorded in this paper were made in the course of investigations on gas gangrene. The work of McIntosh, of Weinberg and Séguin, and of other investigators has shown that the organisms chiefly responsible for the production of gas gangrene are the bacillus of Welch, the *Vibrion septique,* and the *Bacillus œdematiens.* Small amounts of broth cultures of these organisms when injected into animals belonging to a susceptible species, *e.g.*, the mouse or the guinea-pig, produce a violent gas gangrene, and kill the animal within 24 hours.

It is known and was confirmed by us that suspensions in saline of *B. Welchii* and of *Vibrion septique* from a surface culture are practically non-pathogenic; half a cubic centimetre of a dense suspension of these organisms can be injected into a mouse or guinea-pig subcutaneously or intramuscularly without producing gas gangrene and, indeed, without producing any signs of ill-health. The same result is obtained if broth cultures of *B. Welchii* or of *Vibrion septique,* which contain toxins in addition to the bacteria, are centrifuged, and the bacteria, after having been washed free from adherent toxin, are suspended in saline and injected. In the case of *B. œdematiens,* the toxin is so potent that it is not easy to remove the last traces of toxin by washing, and it is necessary to destroy the last traces of toxin by heating the washed bacteria to 80° C. for half-an-hour, when spores are formed. These spores, when suspended in saline and injected, are again non-pathogenic. Very occasionally it does happen that gas gangrene develops after the injection of detoxicated bacteria or their spores, but such an event is quite exceptional and apparently accidental. With the detoxicated *B. Welchii,* for instance, we have observed gas gangrene to occur only once out of a large number of experiments on more than a hundred animals. But if these "detoxicated" bacteria or spores are again mixed with a dose of toxin too small to produce an effect by itself, gas gangrene develops regularly and kills the animal.

Further investigations, which will be published separately, showed that the bulk of the detoxicated bacteria when injected undergoes lysis, while some of the bacteria are taken up by phagocytes and slowly undergo disintegration within the phagocytes, but that these two processes, by means of which the animal defends itself against the infecting bacteria, do not take place when the bacteria are injected together with toxin. It is clear, therefore, that the toxin paralyses the defensive mechanism of the animal. But it also follows that this defensive mechanism is normally so efficient that the formation of toxin in a concentration sufficient to produce this paralysing action is prevented.

We were thus led up to the problem: Why does gas gangrene ever occur in man? Since the defences of the body against the bacteria of gas gangrene are so efficient, the infection of wounds with these bacteria is not in itself an adequate explanation of the occurrence of gas gangrene. And clinical experience leads up to the same problem. Very many wounds, especially those received on the Western Front, were infected with the bacillus of Welch, but only a very small percentage of those infected wounds developed gas gangrene.

It is clear that a new factor, extrinsic to the infection, enters here, and it was in the search for this factor that the phenomenon recorded in this paper was observed and found to have a more general significance.

The small incidence of gas gangrene in wounds infected with the bacteria of gas gangrene is, of course, well known. The explanation generally given is that, owing to the special conditions obtaining in war wounds, the latter offer an exceptionally favourable nidus for the growth of these bacteria. Thus, interference with the circulation, the presence of large masses of dead and lacerated muscle, the presence of cloth, dirt or foreign bodies generally, have all been suggested as factors capable of eliciting gas gangrene. But clinical experience shows that many wounds, in which these factors are undoubtedly present, do not develop gas gangrene, and that there are no clinical features of a wound pathognomonic of gas gangrene. And experiment completely fails to give any support to the nidus theory. Splinters of wood, pieces of muscle, of paper, wool, cotton-wool, and of khaki cloth, have been soaked in a suspension of *B. Welchii*, and then introduced underneath the skin of a mouse without ever producing gas gangrene. Even the minced muscle from a meat broth culture of *B. Welchii* does not produce gas gangrene if introduced subcutaneously after having been freed from toxin by washing with saline. Nor was it found possible to produce gas gangrene by cutting the femoral artery and injecting a suspension of *B. Welchii* into the leg or into the hæmatoma, or by injecting suspensions of *B. Welchii*, together with staphylococci, streptococci, *B. coli*, *B. proteus*, or *B. sporogenes*. The injection of lactic acid in varying concentrations, together with the detoxicated bacteria of gas gangrene, has also failed to produce gas gangrene, even although an extensive sore was frequently produced as the result of the injection of the lactic acid.

The negative results have been reported in detail because they demonstrate clearly how difficult it is to elicit the pathogenic action of the bacteria of gas gangrene or their spores. In this respect they resemble the bacterium of tetanus. We are informed by Dr. Tulloch that, in his experience, it is not possible to induce tetanus regularly and with certainty after infection with the spores by any of the means given in the literature and tested by him. As the negative results of the experiments given above accumulated, the conviction grew that in normal animals, in which the experimental conditions can be controlled and kept constant, an adequate explanation of the relation between these bacteria and the specific disease with which they are associated cannot be deduced from the occasional and apparently accidental production of gas gangrene or of tetanus, as the case may be, which sometimes occurs, but must be based on a definite factor, by which these conditions can be elicited from the bacteria or their spores with certainty and at will. Such a factor was eventually found in injections of small doses of a soluble ionisable calcium salt. The severity of the trauma which can be inflicted on animals infected with the detoxicated bacteria or their spores without eliciting from them the specific disease, stands in striking contrast to the phenomenon which will now be described.

Calcium Salts as an Accessory Factor in Gas Gangrene and Tetanus.

Doses of 2·5 mgrm. of calcium chloride, when injected subcutaneously into mice of 10–15 grm. weight, together with a suspension of a virulent strain of *B. Welchii* or of *Vibrion septique*, will produce a violent gas gangrene in every case. The same dose when injected with a suspension of the tetanus bacillus or its spores will produce tetanus. Larger doses up to 5 mgrm. have the same effect. With mice the dose of calcium chloride cannot safely be increased above 10 mgrm., since with doses of such magnitude the toxic action of calcium salts comes into play. Doses of 10 mgrm. are, as a rule, without any ill effect for normal mice of 15–20 grm. weight. Sometimes, however, even with such a dose the animals are ill a few hours after the injection and die within 24 hours. With smaller doses the effect becomes at first irregular, and if the dose is further diminished fails to appear. The smallest dose with which it has been possible to cause gas gangrene with the spores of *Vibrion septique* has been 0·5 mgrm. of calcium nitrate. For guinea-pigs of 250 grm. weight the minimal dose necessary to produce this effect in every case is larger, namely, 5 mgrm. of calcium chloride. With

B. œdematiens experiments were made only with spores, for the reasons already given, and for these larger doses are necessary to elicit their pathogenic action, namely, 5 mgrm. for a mouse and 10 mgrm. for a guinea-pig. In all our experiments the solutions of calcium salts were used in a strength of 1 per cent. or 2 per cent. It is possible that the dose of calcium salt necessary to produce the effect may vary with the concentration. This point has not yet been tested by us.

For the sake of convenience and brevity it is advisable to designate this new phenomenon by a new name. For reasons which will be given below, and which are based on the mechanism by which the phenomenon is produced, the terms "kataphylaxis," or "defence-rupture," will be used in this paper to describe it. The smallest dose of a calcium salt necessary to elicit the specific disease from the detoxicated bacteria in every case is called the "minimal rupturing dose." Smaller doses which are still capable of eliciting the specific disease, but are not capable of doing so in every case, are called "subminimal rupturing doses." In fixing these doses it is of course assumed that a virulent strain of the specific bacteria is being used. The density of the bacterial suspensions can apparently be varied within wide limits without affecting the results. We have worked, as a rule, with a suspension of such a density that large print is just readable through it in a test-tube. But we have also used denser and more dilute suspensions with practically identical results.

Calcium nitrate and calcium acetate have the same effect as calcium chloride. The insoluble calcium carbonate has no rupturing action. Similar doses of the chlorides of potassium, sodium, ammonium, magnesium, and strontium* have no effect when injected together with a suspension of *B. Welchii.*

B. sporogenes, when injected together with calcium chloride, does not produce gas gangrene, and does not even make the animal ill. It may be recalled that even in broth cultures this organism is non-pathogenic, if present alone.

The rupturing action of calcium chloride is abolished by sodium citrate. As an illustration the following experiment will be given. A number of mixtures of 2 per cent. solutions of calcium chloride and sodium citrate were made up to a volume of 2 c.c. with water. Two drops of a dense freshly prepared suspension of *B. Welchii* in saline were added to these mixtures;

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* [*Note added April* 4.—We have since found that with *Vibrion septique*, the pathogenic properties of which are more readily elicited than those of *B. Welchii,* strontium will produce gas gangrene, if sufficiently large doses (5 mgrm.–10 mgrm.) are given. But, even then, a positive result is not obtained in every animal, as is the case with *B. Welchii.*]

0·5 c.c. of each of these mixtures were injected subcutaneously into batches of three mice. The results were as follows :—

Batch.	$CaCl_2$ solution 2 per cent.	Na citrate solution 2 per cent.	H_2O.	Dose of $CaCl_2$ injected.	Mouse 1.	Mouse 2.	Mouse 3.
	c.c.	c.c.	c.c.	mgrm.			
A	1	—	1	5	+	+	+
B	1	0·5	0·5	5	+	+	+
C	1	1	—	5	0	0	0
D	0·5	—	1·5	2·5	+	+	+
E	0·5	0·5	1	2·5	0	0	0
F	0·5	1·5	—	2·5	0	0	0

In the Tables + means "died," 0 means "alive and well."

All the mice in batches A, B, and D developed gas gangrene and died within 20 hours after the injection, except one animal in batch D, which died within 48 hours. All the animals in batches C, E, and F remained alive and well for three days after the injection.

By means of a similar experiment it has been possible to demonstrate an antagonism between Ca ions and Mg ions with reference to the production of gas gangrene from *B. Welchii.* 0·2 c.c. of a suspension of these bacteria was added to mixtures of equimolecular solutions of $MgCl_2$ and $CaCl_2$ in varying proportions. These mixtures were then injected subcutaneously into batches of four mice each. In each batch two mice received a "rupturing dose" of calcium chloride, *i.e.*, a dose which contained 2·75 mgrm. of $CaCl_2$, two mice received a "subminimal rupturing dose," containing 1·4 mgrm. of $CaCl_2$, *i.e.*, a dose which does not elicit gas gangrene in every case. The results are as follows :—

Batch.	$CaCl_2$ solution M/5.	$MgCl_2$ solution M/5.	H_2O.	Dose of $CaCl_2$ injected.	Result after 3 days.	
					Mouse 1.	Mouse 2.
	c.c.	c.c.	c.c.	mgrm.		
A	0·5	—	1·5	2·8	+	+
B				1·4	+	+
C	0·5	0·5	1	2·8	+	+
D				1·4	0	0
E	0·5	1	0·5	2·8	+	+
F				1·4	0	0
G	0·5	1·5	—	2·8	+	0
H				1·4	0	0

The experiment shows a distinct protective action of the magnesium salt against a subminimal rupturing dose of the calcium salt, when tested with *B. Welchii.*

In all the experiments mentioned so far, the bacteria or their spores have been injected suspended in the various salt solutions. This direct contact between the bacteria and the calcium salts gives the most favourable conditions for the phenomenon which we have described, but it is not essential. It has been possible to produce gas gangrene in mice by injecting the spores of *Vibrion septique* and calcium salts either at the same site at different times, or secondly, at the same time at different sites, the injection of calcium salts either preceding or following that of the spores. Similarly, tetanus has been produced by injecting the spores and the calcium salt at different sites at the same time and also by injecting calcium salts and tetanus spores at the same site but at different times, the injection of spores also either preceding or following that of the calcium salts. In such an experiment, for instance, no tetanus occurred for seven days in mice which had received an injection of tetanus spores. But, when, on the seventh day, calcium chloride was injected tetanus developed on the following day.

These experiments, in which the injections of calcium salts and of bacteria were separated in time or in space, throw light on the mechanism by which the peculiar effect of calcium salts, with which this paper deals, is brought about. A detailed consideration of these experiments will be given below in dealing with the probable explanation of the phenomenon.

On the Etiology of Gas Gangrene and of Tetanus.

We have referred in the introduction to the fact that infection with the bacteria of gas gangrene and of tetanus is not in itself an adequate explanation of the specific diseases produced by these bacteria, and that it was necessary to postulate the existence of an accessory factor. The question which naturally suggests itself is whether the phenomenon which we have described in this paper represents this accessory factor.

It has always been realised that both tetanus and gas gangrene are diseases particularly associated with earth, and, in fact, these diseases can be produced by injecting emulsions of earth into mice or guinea-pigs. The explanation which is generally accepted is that the soil contains the spores of the specific bacteria. No specific action is credited to the earth itself; it is supposed to act as a foreign body like many other things, such as pieces of cloth, or splinters of wood, the presence of which is assumed to create an exceptionally favourable nidus for the growth of the bacteria. As stated in the introduction we have failed to find any experimental evidence which would support the nidus theory. Vaillard,* who investigated in great detail the relation of the contamination of the wound with earth to the etiology of tetanus, and whose observations as to the inadequacy of the nidus theory agree in many respects with ours, recognised that this contamination exercises a specific influence. But he ascribed this specific factor to the presence of other micro-organisms in the earth.

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* Vaillard et Rouget, 'Annales de l'Institut Pasteur,' vol. 6, p. 385 (1892).

Of the various foreign bodies tested by us, emulsions of earth were the only ones which frequently, although not invariably, elicited gas gangrene when injected together with the detoxicated bacteria of gas gangrene or their spores. That the effect of earth is not due to its mechanical action but to a chemical constituent of the earth was demonstrated by the following experiment: A sample of earth was taken which, when autoclaved and made into an emulsion, would elicit gas gangrene from the spores of *Vibrion septique.* The watery extract of such an emulsion, after having been filtered through filter paper, autoclaved and tested for its sterility, was just as capable of eliciting gas gangrene as the original emulsion. It could be shown, moreover, by qualitative chemical tests, that this earth extract contained calcium salts and that it lost its power to elicit gas gangrene when these salts had been removed by precipitation with sodium carbonate. We are not however prepared to state that the calcium salts in the soil are the only chemical constituents which are responsible for this phenomenon, for some extracts of earth which were capable of eliciting gas gangrene from the spores of *Vibrion septique* contained only traces of calcium salts. It seems probable that some other chemical substance present in these extracts, which also forms an insoluble carbonate, is also capable of producing the kataphylactic phenomenon, and may be even more powerful in this respect than calcium salts. Further investigations on this point are being carried out.

It is of interest to note that samples of earth taken from the surface may fail to show this effect when a sample from the same locality about 6 inches below the surface will give a positive result. It has also been found that samples from different localities differ in their activity, and that samples taken from the same locality and from the same depth may show differences at different times of the year, *i.e.*, according to the treatment, such as "liming," which the soil has received. These differences may not exhibit themselves qualitatively by the presence or absence of the power to elicit gas gangrene or tetanus, but quantitatively by the doses of earth extract or emulsion necessary to produce this effect. These observations account satisfactorily for the curious fact that the occurrence of gas gangrene on the Western Front was very "patchy." It varied with the locality in which the wounds had been received, and was relatively infrequent in certain localities, even although the wounds were infected with the bacteria of gas gangrene.

There can be little doubt that the presence of certain simple chemical constituents of the soil which have the property of producing the kataphylactic phenomenon is responsible for the occurrence of gas gangrene and of tetanus. This statement does not imply that every case of gas gangrene and of tetanus can be accounted for in this way. Experimentally, mice infected with detoxicated *B. Welchii* and exposed to cold have occasionally developed gas gangrene. As a rule, gas gangrene does not develop in mice exposed to cold either before or after the injection of a suspension of *B. Welchii.* But out of 16 mice, two animals, in which the exposure to cold had been exceptionally severe so that the animals were already ill when they received the injection of *B. Welchii,* did develop gas gangrene. The experiments with cold are complicated by the fact that mice frequently develop an enteritis as the result of a severe exposure to cold. The general depression of the vitality of an animal which has received such a severe exposure to cold may be reasonably assumed to involve also the processes of lysis and phagocytosis which constitute the defensive mechanism of the infected animal against the infected bacteria, and thus account for the development of gas gangrene in the two positive experiments. In the human subject, where such a depression may be the result not only of exposure to cold but also to shock, it will probably be responsible also for the development of gas gangrene in some cases. But it cannot be looked upon as the only or even most frequent exciting cause, since gas gangrene develops in men who, apart from the wound, are in good health, are not in a state of shock, and have not been exposed to cold.

There is another way in which tetanus and gas gangrene can be produced experimentally in animals infected with the detoxicated bacteria without making use of the phenomenon of defence rupture. Tulloch* has shown that tetanus spores will produce tetanus in guinea-pigs if they are injected together with a non-lethal dose of the toxin of *B. Welchii.* We have found that gas gangrene can be produced in mice if the detoxicated *B. Welchii* are injected together with diphtheria toxin, which happens to be non-lethal for mice but has a transient local action. The explanation of this fact is probably to be found in the aggressin-like nature of the toxins. We have pointed out in the introduction that the toxin of *B. Welchii* acts at first by paralysing the defensive mechanism of lysis and phagocytosis by which the animal defends itself against infection with the detoxicated *B. Welchii,* and diphtheria toxin has probably a similar effect in mice. It seems possible, therefore, that the presence of a non-specific and non-lethal toxin with an aggressin-like action may have to be considered as a factor in the causation of gas gangrene or of tetanus. As stated in the introduction, we have not been able to obtain experimental evidence in support of this view in the case of gas gangrene by injecting the bacteria of gas gangrene together with other bacteria which are likely to form concomitant infections in wounds. And even in the production of tetanus from the spores by means of the toxin of *B. Welchii*, it must be remembered that this does not represent the effect produced by a concomitant infection with the bacilli of Welch. For such an infection does not, as we have seen, lead to the production of toxin sufficient to paralyse the defensive mechanism. It still requires to be demonstrated that tetanus will result when the spores of tetanus are injected together with the detoxicated bacteria of Welch.

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* Tulloch, 'British Medical Journal,' June 1, 1918.

The preceding considerations emphasise the importance of the contamination of the wound with earth from the point of view of the etiology of gas gangrene and of tetanus. For this contamination carries into the wound not only the infecting bacteria, but also frequently the chemical constituents of the soil capable of producing the phenomenon of defence-rupture. But there is also some experimental evidence to show that tetanus and gas gangrene may sometimes develop even without the intervention of the phenomenon.

On the Mode of Action of Calcium Salts.

Two possible explanations suggest themselves at once: the calcium salts may produce their effect either by making the bacteria more virulent, or by making the animal more sensitive to the action of the bacteria. Both these possibilities were tested experimentally.

In order to see whether there is a direct action on the bacteria, *B. Welchii* freed from toxin were suspended in a solution of calcium chloride and incubated for three hours. The suspension was then centrifuged, and the bacteria after washing with saline suspended in a sodium chloride solution, and the suspension injected into mice. No effect was produced.

The same negative result was obtained when *B. Welchii* were incubated for 20 hours in a broth culture to which some calcium chloride solution had been added. A suspension of *B. Welchii* in saline prepared from such a culture was incapable of producing gas gangrene. It is clear, therefore, that calcium salts do not act directly on the bacteria in such a way as to render them capable of producing gas gangrene by themselves in the absence of a toxin. Nor does the addition of calcium chloride to a broth culture increase the amount of toxin produced. This was demonstrated both for *B. Welchii* and for *Vibrion septique*. It was however noted that the rate of growth of *B. Welchii* and of *Vibrion septique* was much more rapid after the addition of calcium chloride. It was further found that a culture of *B. Welchii* or

Vibrion septique, obtained by inoculating meat broth with fragments of breast muscle from pigeons which had been inoculated four hours previously with a broth culture of these organisms to which calcium chloride had been added, was not more virulent than a culture from a pigeon which had been infected with broth cultures alone of these bacteria.

It is clear, therefore, that the action of calcium chloride is not due to a change in the essential properties of the bacteria so as to make them individually more virulent. This conclusion is confirmed by the observation, to which reference has already been made, that a direct contact between the bacteria and calcium salts is not essential for the production of the kataphylactic effect. There remains the other alternative, that the effect of calcium salts might be to make the animals more sensitive to the action of the bacteria. Now it is known that the bacteria of gas gangrene and of tetanus owe their pathogenic effect to the formation of a toxin. The correctness of the second alternative can therefore be tested by determining whether the injection of calcium chloride diminishes the minimal lethal dose of these toxins. This test was applied to the toxin of *B. Welchii*. It was found that the injection of calcium salts did not make an animal more sensitive to the action of this toxin. What, then, is the explanation of the phenomenon described in this paper if it cannot be attributed either to an increased sensitiveness of the infected animal or to an increased virulence on the part of the infecting bacteria? A partial answer to this question is given by the experiments, in which the injections of calcium salts and of bacteria were separated in time or in space.

Gas gangrene will result if *Vibrion septique* spores are injected into an animal which some hours or days previously has received an injection of calcium salts. This result is obtained most readily if the two materials are injected into the same site, and in that case the interval between the two injections may extend over several days if a large dose of calcium salt has been given. Thus in two different experiments the following results were obtained: 2·5 mgrm. Ca salt given two hours before *Vibrion septique* spores: injected into same site into eight mice. Result.—All mice dead within 24 hours. 10 mgrm. Ca salt given three days before *Vibrion septique* spores: injected into same site into nine mice. Result.—All mice dead within 24 hours. Even if the two materials are inoculated in two different sites, gas gangrene may follow, although not in every case.

The following experiment may be given to illustrate the result of separating the sites and times of injecting the bacteria and the rupturing substance. In this experiment 36 mice, in 12 batches of 3 mice each, received at the same time injections of calcium nitrate in varying doses.

At two different intervals of time—2 and 24 hours afterwards—the mice received injections of a suspension of *Vibrion septique* spores, half the number of animals being injected subcutaneously at the same site (right flank), the other half being injected subcutaneously on the back. The results were as follows:—

Gas Gangrene from *Vibrion septique.* Effect of separating Time and Site of Injections of Bacteria and of Calcium Salt.

Batch.	No. of mouse.	Dose of Ca nitrate.	Interval of time.	Site.	Result after 24 hours.
		mgrm.	hours.		
A	1 2 3	2·5	1¼	Same	+ + +
B	4 5 6	2·5	1¼	Different	0 0 0
C	7 8 9	2·5	20	Same	+ + +
D	10 11 12	2·5	20	Different	0 0 0
E	13 14 15	5	1¼	Same	+ + +
F	16 17 18	5	1¼	Different	0 + +
G	19 20 21	5	20	Same	+ + +
H	22 23 24	5	20	Different	0 0 0
J	25 26 27	10	1¼	Same	+ + +
K	28 29 30	10	1¼	Different	+ 0 0
L	31 32	10	20	Same	+ +
M	33 34	10	20	Different	0 0
Control	35 36	Spores alone	—	—	0 0

This experiment shows that a minimal rupturing dose will elicit gas gangrene in every case from the spores of *Vibrion septique*, even when the latter are injected after an interval of 20 hours into the same site. When different sites are used, however, the production of gas gangrene becomes more difficult, and it is necessary to use larger doses of the calcium salt and to inject the spores within a short time after the calcium salts. *Post-mortem* examinations were made of one dead mouse in each batch. All showed the typical lesion of *Vibrion septique* gas gangrene in the right flank, and this was the case even in the three mice where the spores had been injected on the back. Only one of these three mice had the typical lesion also on the back; the two others only showed a slight serous exudate, such as is found in normal mice after the injection of spores. This remarkable fact will be referred to again below.

Similar experiments were carried out with a suspension of *B. Welchii.* With these bacteria the dose of calcium salt has to be increased to 5 mgrm. if an interval of time is allowed to elapse before the injection of the bacteria, and this interval of time within which gas gangrene can be produced is limited to a few hours. After an interval of 24 hours, we have not been able to produce gas gangrene even with a dose of 10 mgrm. of calcium salt. Doses of 10 mgrm. of calcium salt are necessary when different sites are used. Under these conditions, we have observed two different courses which the infection may take. Either, as in the case of *Vibrion septique*, the typical lesion of *B. Welchii* gas gangrene is produced at the site of injection of the calcium salt, and may be completely absent from the site where the bacterial suspension was introduced; or there is no distinct localised gas gangrene lesion, but a *B. Welchii* septicæmia results, in which the hæmolytic action of the infection seems to predominate. For in such animals *B. Welchii* can be seen in films made from the heart blood, the liver and kidney are very pale instead of showing the intense congestion of a typical *B. Welchii* gas gangrene, and the blood is very poor in hæmoglobin.

Experiments with spores of *B. tetani* (Type II of Dr. Tulloch) gave essentially the same results as those with *B. Welchii* and the spores of *Vibrion septique.* As the Table shows, it was possible to elicit tetanus by injecting the spores 2½ hours after the injection of even the minimal rupturing dose of the calcium salt, when the spores were injected into the same site as the calcium salt. But, when different sites were used, tetanus did not occur even with the biggest dose of calcium salt used. Even with an interval of 24 hours tetanus was produced, but then it was delayed and occurred only with larger doses of calcium salt and using the same site. It must be noted, however, that for the experiments with a 24-hours' interval, a different and older preparation of spores was used, which appeared to have lost some of its virulence, since the spores did not produce tetanus in every case when injected together with a rupturing dose of a calcium salt.

The details of the experiment are given in the following Table. It is perhaps necessary to point out that this experiment is quite different from those mentioned in an earlier part of this paper, in which tetanus spores were injected first and then calcium salts.

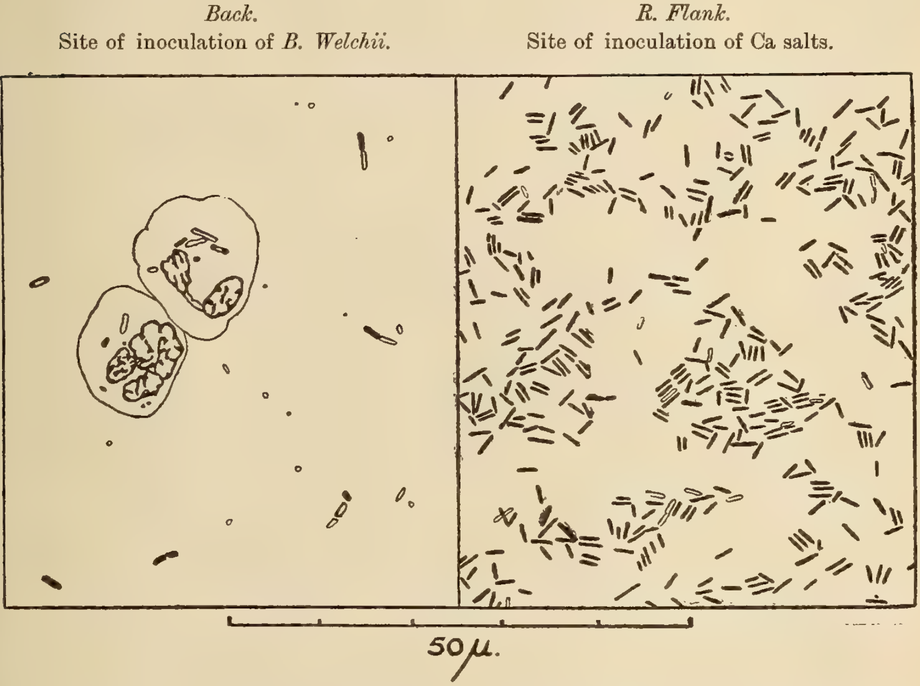
Tetanus. Effect of separating Time and Site of Injections of Bacteria and of Calcium Salt.

Batch.	No. of mouse.	Dose of Ca nitrate.	Interval of time.	Site.	Result after 1	2	3	4 days.
		mgrm.	hours.					
A	1	2·5	2½	Same	0	0	0	0
	2				0	+	—	—
B	3	2·5	2½	Different	0	0	0	0
	4				0	0	0	0
C	5	5	2½	Same	0	+	—	—
	6				0	+	—	—
D	7	5	2½	Different	0	0	0	0
	8				0	0	0	0
E	9	10	2½	Same	0	+	—	—
	10				0	+	—	—
F	11	10	2½	Different	0	0	0	0
	12				0	0	0	0
G	13	5	24	Same	0	0	0	+
	14				0	0	0	0
H	15	5	24	Different	0	0	0	0
	16				0	0	0	0
J	17	10	24	Same	0	+	—	—
	18				0	0	+	—
K	19	10	24	Different	0	0	0	0
	20				0	0	0	0
Control	21	Spores alone	—	—	0	0	0	0
	22				0	0	0	0
	23				0	0	0	0
	24				0	0	0	0
	25				0	0	0	0
	26				0	0	0	0

0 means alive and well with absence of symptoms of tetanus. + means died with symptoms of tetanus.

All these experiments point to the conclusion that the injection of calcium salts produces at the site of injection a local change in the tissues of the animal since the specific disease is most easily elicited when the calcium salt and the bacteria are injected into the same site. It does not appear reasonable to suppose that this local effect is due to the actual presence of calcium salts at the site of injection. Since the effect is produced only by soluble, ionisable calcium salts, it would be necessary to assume that such a salt could remain deposited at the site of injection for a day or even three days. Moreover, if that were so, it ought to be possible to counteract their effect by injecting sodium citrate together with the bacterial suspension. That, however, we have been unable to do. In one experiment, for instance, eight mice received an injection of 2·5 mgrm. of calcium chloride in the right flank. Two hours afterwards four of the mice received a suspension of *Vibrion septique* spores in saline in the right flank, while the other four received a suspension of these spores in 2 per cent. sodium citrate solution, also in the right flank. All the eight mice were dead within 24 hours with the typical local lesion of gas gangrene. Similar experiments have been carried out with the same result. Sodium citrate only protects when it is mixed with the calcium salt before it is injected into the animal.

One must conclude, therefore, that calcium salts produce a local change in the tissues at the site of injection. This conclusion is confirmed by the fact that it is much more difficult to produce gas gangrene and tetanus when the bacteria and the calcium salts are injected at different sites, than when they are injected at different times. The local change produced in the tissues by calcium salts persists for a considerable time, and has, as the ultimate result, the formation in the animal of a place of diminished resistance against the infecting bacteria. The most striking confirmation of this conception is furnished by the result of the experiments in which gas gangrene occurred after injection of the calcium salt and bacterial suspension at different sites. For in these experiments the fact was observed that the typical local lesion of gas gangrene—an intense hæmorrhagic œdema—occurs always at the site of injection of the calcium salt, while the site where the bacteria have been injected does not show, as a rule, any macroscopic evidence of gas gangrene. Films made from the two sites present this difference very strikingly (see fig. 1); the film from the site of injection of *B. Welchii* shows active lysis, as indicated by the presence of bacterial *débris*, and active phagocytosis with intracellular digestion of the engulfed bacteria. Only a few bacteria can still be seen lying free and apparently intact, but many of these have become gram-negative. The picture is practically identical with that given by a normal mouse which has received a suspension of detoxicated bacteria, and which is defending itself successfully against the infection. But the film from the site of injection of the calcium salt presents the picture typical of an animal which has developed gas gangrene by, let us say, the injection of the bacteria together with toxin. The whole field is covered with densely packed bacteria which are almost all gram-positive. Only very few leucocytes can be seen, and none of these show any phagocytosis.



Back.
Site of inoculation of *B. Welchii.*

R. Flank.
Site of inoculation of Ca salts.

FIG. 1.—Films from the back and right flank of a mouse which developed gas gangrene and died 24 hours after having received first 10 mgrm. of $CaCl_2$ in the right flank, and 2 hours later a suspension of *B. Welchii* in saline on the back. Stained with gram and neutral red. Gram-negative staining is indicated by merely outlining the bacteria.

Both macroscopically and microscopically the picture presented by the right flank is that of an animal which has succumbed to the *B. Welchii* infection; the picture presented by the back that of an animal which is successfully defending itself against such an infection.

This apparently paradoxical result becomes clear in the light of the conception that the calcium salts produce at the site of their injection a local change in the tissues which has as its ultimate effect a local breaking down of the defensive mechanism. This conception of the mechanism is expressed by the terms "defence rupture" or "kataphylaxis," with which we have designated this phenomenon.

When calcium salts and bacteria are injected together it is quite possible that this effect may be assisted by other factors. There may be a direct favouring effect of the calcium salts on the rate of growth of the bacteria, such as has been observed *in vitro*. It is conceivable too that calcium salts may directly interfere with phagocytosis and lysis, and experiments *in vitro* will have to be carried out in order to test this point. But these factors, even admitting for the sake of the argument that calcium salts did produce these effects *in vivo*, could only be accessory factors under the special experimental conditions which involve the actual presence of calcium salts. When a separation of the sites or times of the injections is made, the actual presence of calcium salts can be excluded, but the phenomenon still makes its appearance.

Summary.

The bacteria of gas gangrene (*B. Welchii*, *Vibrion septique*, and *B. œdematiens*) and of tetanus, when completely freed from their toxins, either by washing or by heating to 80° C. for half-an-hour, so that spores are formed, do not produce the specific disease when injected into a mouse or a guinea-pig. The normal animal disposes of the bacteria mainly by lysis and partly also by phagocytosis, and this defensive mechanism is so efficient as to render these bacteria non-pathogenic when injected by themselves.

If a small dose of a soluble ionisable calcium salt is injected together with the bacteria or their spores, the specific disease is elicited in a very virulent form. The chlorides of sodium, potassium, ammonium, strontium and magnesium, when injected together with *B. Welchii*, are not capable of producing gas gangrene.

A direct contact between the bacteria and the calcium salt is not essential. The phenomenon will occur if the bacterial suspension and the calcium salt are injected at different times into the same site, or into different sites at the same time or at different times.

From these experiments and other experimental evidence the conclusion is drawn that calcium salts, when injected subcutaneously, produce a local change in the tissues at the site of injection. The effect of this change is to bring about a local breaking down of the defensive mechanism against the bacteria of gas gangrene and tetanus. The terms "kataphylaxis" or "defence rupture" are proposed to designate this new phenomenon.

Sterile watery extracts of earth are capable of producing this phenomenon. They may owe this property in many cases entirely to the presence of calcium salts, but there is evidence that in some cases the extracts of earth owe their rupturing action to the presence of another chemical substance or substances which have not yet been identified.

The bearing of these observations on the etiology of gas gangrene and tetanus is discussed.

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BIOLOGICAL SCIENCES.

CONTENTS.

Page

The Distribution of the Serological Types of B. tetani in Wounds of Men who received Prophylactic Inoculation, and a Study of the Mechanism of Infection in, and Immunity from, Tetanus. By Major W. J. TULLOCH, M.D., R.A.M.C., Lecturer in Bacteriology, University of St. Andrews, Member of the War Office Committee for the Study of Tetanus . . 529

The Existence of Daily Growth-rings in the Cell Wall of Cotton Hairs. By W. LAWRENCE BALLS, M.A., Sc.D. (Cantab.), late Fellow of St. John's College, Cambridge. (Plates 14–16) 542

CROONIAN LECTURE.—The Biological Significance of Anaphylaxis. By Dr. H. H. DALE, F.R.S. 556

Obituary Notices of Fellows deceased :—Henry George Plimmer (with portrait) ; Alfred Merle Norman (with portrait) . . . . . xliii—xlix

Index . . . .

Title, Contents, etc.

PRINTED AND PUBLISHED FOR THE ROYAL SOCIETY BY
HARRISON & SONS, 44—47, ST. MARTIN'S LANE, LONDON, W.C. 2.
Price Four Shillings and Sixpence.

No. B 634.

July 1, 1919.

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The figure is by Mr. W. Pilgrim, the laboratory draughtsman. We wish to express our great indebtedness to Dr. H. Henry, Dr. J. McIntosh, Dr. R. A. O'Brien, Miss Muriel Robertson, and Captain W. J. Tulloch, R.A.M.C., for their readiness in assisting our investigations by supplying us with information and material.

The Distribution of the Serological Types of B. tetani *in Wounds of Men who received Prophylactic Inoculation, and a Study of the Mechanism of Infection in, and Immunity from, Tetanus.*

By Major W. J. Tulloch, M.D., R.A.M.C., Lecturer in Bacteriology, University of St. Andrews, Member of the War Office Committee for the Study of Tetanus.

(Communicated by Major-General Sir David Bruce, F.R.S. Received January 30, 1919.)

(From the Laboratories of the Lister Institute of Preventive Medicine and the Royal Army Medical College.)

Introduction.

The bacteriological investigation of tetanus, the results of which are summarised in the present communication, was undertaken on behalf of the War Office Committee for the Study of Tetanus.

Before the bacteriological examination of a relatively large number of cases of tetanus could be effected, it was found necessary to elaborate a suitable technique,* as the existing methods for the cultivation of *B. tetani* proved to be wholly inadequate.

Apart from its practical application, this preliminary work was of great value, in that it called attention to the important factor of symbiosis in the growth of anaërobic bacteria. The cycle in the development of anaërobic bacteria in cultures of the mixed flora of wound exudates is remarkably constant, and it is not improbable that a similar sequence occurs in the wounds themselves. The first organisms to appear are rapidly growing bacilli typified by *B. Welchii*, followed by the proteolytic group of anaërobes of the *B. sporogenes* Types. On the decline of the proteolytic group, *B. tetani* and other similar organisms appear, which may ultimately predominate in the cultures.

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* See p. 539.

These preliminary inquiries also resulted in the differentiation of three serological Types of *B. tetani* (1) and (2). The demonstration of these Types raised the following questions :—

(i) Does the administration of anti-toxin corresponding to one Type confer immunity against all Types ?

(ii) If this be the case, is the immunity so conferred more adequate against *homologous* bacilli than against serologically *heterologous* bacilli ?

These questions will be dealt with under the following headings :—

Section I.—Inquiry into the prevalence of the various Types of *B. tetani* in wound exudates of cases of declared tetanus, together with a corresponding investigation of wounds of men showing no evidence of tetanus.

Evidence of geographical distribution of types of tetanus bacilli.

Section II.—Examination of the mechanism of *infection* in tetanus as distinct from the mechanism of *intoxication.*

Section III.—Serum prophylaxis in experimental animals from the standpoint of infection.

Section IV.—Inquiry as to whether

(*a*) Monotypical anti-toxin exhibits specific anti-infective properties.

(*b*) Inoculations of cultures of *B. tetani* give rise to anti-bacterial bodies specific to the type of organisms used.

SECTION I.

(A) *Prevalence of the Different Types of* B. tetani *in Wound Exudates from Declared Cases of the Disease as Differentiated by the Agglutination Method.*

(B) *Prevalence of the Different Types of* B. tetani *in Wounds of Men showing no Evidence of Tetanus Differentiated as in* (A).

A. Attention must be drawn to the fact that the cultures used in the preparation of sera employed in these investigations were subjected to very critical scrutiny before they were used as "Type Strains." They were purified with extreme care, the final "isolation" being effected by Barber's micro-inoculation method (3). I might here point out that over a period of at least 20 months the stock cultures have remained consistently true to Type, and that even with sera having a titre of more than 1/3000 the agglutination reactions have remained specific.

In the early stages of the investigation it was found that all the strains of *B. tetani* obtained from the various Serum Institutions in this country agglutinated with the serum prepared against U.S.A. standard strain. This strain will hereafter be referred to as "Type I." It was therefore of paramount importance that a census should be made of the various tetanus bacilli recovered from cases of the disease. Such an investigation would give an indication as to whether in man a monotypical serum gives adequate protection against all Types. If—either in a qualitative or quantitative sense—such protection were specific, the preponderance of cases among inoculated men should prove to be due to infection with heterologous Types.

The first series of 14 toxic cultures investigated by the agglutination technique were all obtained from wounds of men who had received serum prophylaxis.

The methods used in the examination of this series are fully dealt with in previous publications, but the results may be summarised as follows:—

Twelve of the 14 cultures examined reacted in the presence of one or other of the Type agglutinating sera; 2 proved to be Type I, 7 Type II, and 3 Type III. One of the two remaining cultures was found, on further purification, to contain Type II, but the other could not be placed. It is probable, in the latter case, that the number of tetanus bacilli in the culture was small, for when mice and rats were injected with as much as 0·5 cub. cm. doses of a 4-day culture, only local tetanus developed, and that four days after inoculation.

Agglutinating sera, prepared by the inoculation of non-toxic end-sporing bacilli (some of which closely resemble *B. tetani*), failed to agglutinate relatively pure toxic cultures. Further, the type sera failed to flocculate cultures of non-toxic end-sporing bacilli.

A second series of investigations were then undertaken, and strains from 100 cases of declared tetanus occurring among wounded men have been successfully examined by the agglutination technique. During this inquiry a fourth type of *B. tetani* appeared, but it has only been found to occur in four cases of the disease.

The distribution of the types in this series is given in Table I.

Table I.—Distribution of Types in cases of Declared Tetanus.

Number.	Type.	Percentage.
41	I	41
22	II	22
33	III	33
4	IV	4

The actual incidence and death-rate percentage from each "type infection" is graphically shown in fig. 1.

Of this series of 100 cases, full information was available concerning 91 men who were known to have received prophylactic treatment. The serum used for prophylaxis was presumably prepared mainly from growths of Type I bacilli.

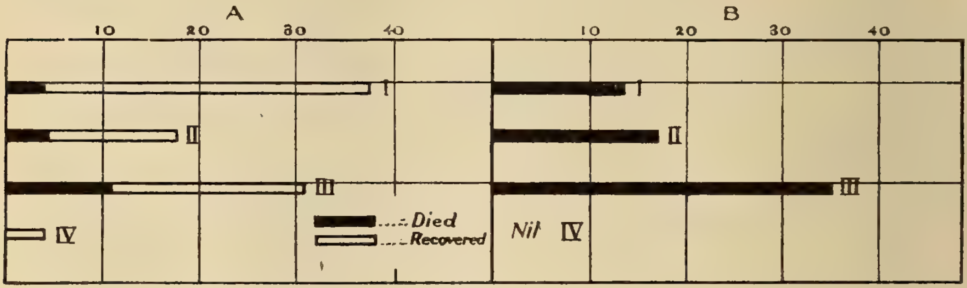

Fig. 1.—Declared Cases of the Disease occurring in 91 Inoculated Men.

If the consideration be limited to those cases of the series in which tetanus supervened within 14 days, the results obtained may be set forth graphically as in fig. 2.

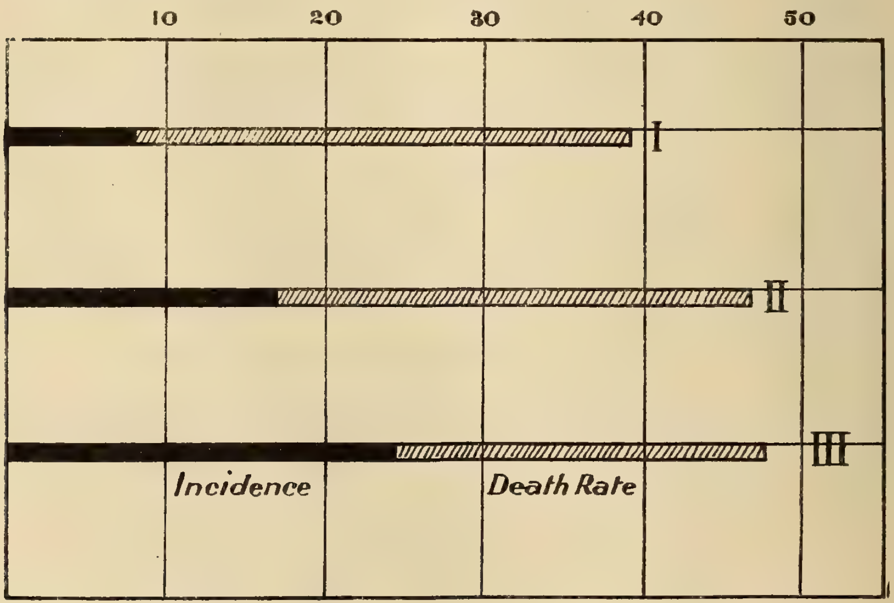

Fig. 2.—Cases of Tetanus in Inoculated Men due to Types I, II and III, in which the onset occurred within two weeks. Rates expressed in percentages.

Summary.—These findings thus suggest that prophylaxis with monotypical anti-toxin may possibly result in monotypical immunity.

(B) On making a similar inquiry into the prevalence of different serological types of *B. tetani* in wounds of men *showing no evidence of tetanus*, a number of important points were brought out.

(i) In a series of 100 wounds, *B. tetani* was recovered and typed in 19 instances. The distribution of the Types was as follows:

Fifteen contained Type I bacilli; two contained Type II bacilli; one contained Type III bacilli; one contained Type IV bacilli.

The method adopted was, if possible, to obtain five swabs from each wound at intervals of approximately one week. In the 19 cases in which *B. tetani* was found, the organism was not invariably present in all five swabs. This can be readily understood when it is borne in mind that the infection may be localised to a particular area in the granulating surface. This is, in fact, the same problem as that which is met with in making statistics of diphtheria carriers; but in the case under consideration, the error is magnified owing to the technical difficulties of the investigation.

(ii) The persistence or recrudescence of tetanus infection of wounds is remarkable. This is well exemplified in the present series by one case especially, in which the bacillus was recovered 882 days after injury.

(iii) In those cases of the series in which *B. tetani* was demonstrated in more than one swab, the organism recovered on each occasion remained true to type.

(iv) In all, 25 strains of *B. tetani* have been obtained from non-tetanus cases. This includes the 19 instances above mentioned, together with six specimens sent for investigation from various sources.

The results are given in the following Table :—

Table II.—Distribution of the Different Types of *B. tetani*, obtained from wounds of men showing no evidence of Tetanus.

Number.	Type.	Percentage.
19	I	76
3	II	12
2	III	8
1	IV	4

If, now, Tables I and II be compared it will be seen that:

(i) If the total number of cases in Tables I and II be taken together, the number of infections with Type I is large, but the percentages of declared tetanus and of the death rate, on the other hand, are low.

(ii) In the case of infections from Types II and III respectively, the total numbers of infections is not so large as in the case of Type I, but the percentage incidence of the disease among men infected is higher, and the death rate is also considerably higher.

These facts are graphically shown in fig. 3.

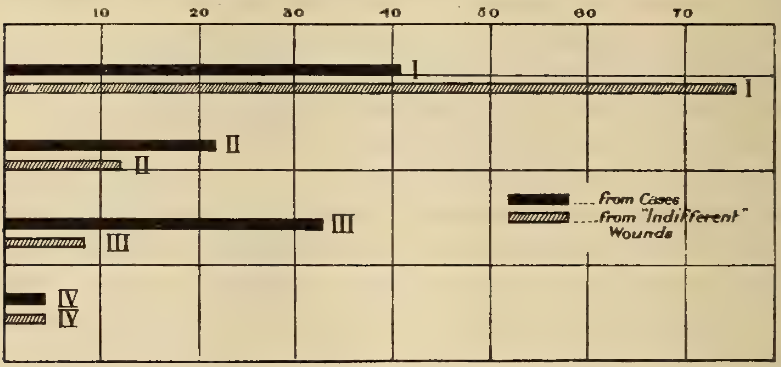

FIG. 3.—Types of *B. tetani* isolated from cases of the disease compared with those isolated from "indifferent wounds," expressed as percentage of the total numbers isolated from each source.

If the figures of Table II be considered as an index of the prevalence of the various Types, it can be assumed that, of x wounded men, one falls a victim to tetanus; it then follows that the number of wounded men who will have been infected with Type I bacilli before one case of the disease occurs will be $15/100 \times x$. Similarly, Type II will be $2/100 \times x$, and Types III and IV $1/100 \times x$. This is indicated graphically in fig. 4.

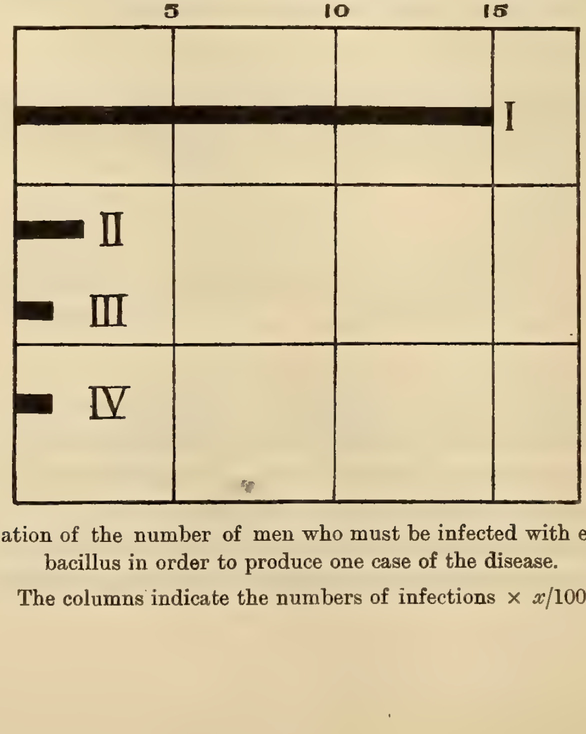

FIG. 4.—Estimation of the number of men who must be infected with each type of the bacillus in order to produce one case of the disease.

The columns indicate the numbers of infections × $x/100$.

Summary.—This Section of the investigation suggests that the serological Type of the infecting bacillus may be of importance in relation to the pathogenesis of tetanus in men who have received serum prophylaxis. Proof of such relationship could, however, only be obtained by the investigation of a large number of cases of the disease. Other methods of inquiry, therefore, had to be undertaken. These are dealt with in Section IV of this communication.

Evidence of Geographical Distribution of Types of B. tetani.

When the first series of 14 toxic cultures was under examination, fighting was especially heavy in the northern portion of the British front; and it was during this period that Type II bacilli were recovered with the greatest frequency. With the later fighting on the Amienois Sector, Types I and III predominated. But shortly before the cessation of hostilities, when severe fighting again occurred in the northern area, the number of Type II infections again increased. These later cases are not included in the series dealt with in this communication, as full details concerning them were not available at the time of writing.

SECTION II.

Mechanism of Infection in Tetanus.

In investigating this question, the following points were noted:—

(*a*) Bacilli or spores deprived of their growth products by washing, or by washing and heating combined, failed to produce the disease in susceptible animals, even when injected in very large numbers—2000 million in the case of guinea-pigs and 200 million in the case of mice.

(*b*) Tetanus toxin, when injected into rats in a sub-lethal dose, together with *washed* tetanus bacilli, fails to induce the growth of the organism. The aggressive qualities, therefore, of tetanus toxin, as ordinarily prepared, are not marked. In this respect the pathogenesis of tetanus differs considerably from that of gas gangrene.

(*c*) When the toxin of *B. Welchii* is inoculated into animals in much less than the lethal dose, together with the spores of *B. tetani*, it does not determine the development of these spores, and therefore the onset of tetanus.

The same may be said with regard to the toxin of *Vibrion septique*, but the results are not so constant.

Serum which is anti-toxic to the products of *B. Welchii* will, in such circumstances, prevent the development of tetanus infection—if used sufficiently soon after the administration of the infecting *inoculum*.

(*d*) Somewhat unexpected results were obtained when chemical irritants were used to produce the necessary local tissue debilitation required to set up tetanus infection.

The chemicals used were—

(1) lactic acid; (2) trimethylamine; (3) saponine; (4) calcium chloride.

(1) *Lactic Acid.*—This gave very uncertain results.

(2) *Trimethylamine.*—In certain concentrations this reagent, together with the spores of *B. tetani*, not infrequently sets up infection in guinea-pigs, but seldom does so in mice. It was found that, in mice, the formation of a large eschar resulted from the injection of this reagent in sufficient concentration, together with 200 million tetanus spores, without, however, inducing tetanus, although the eschar ultimately separated from the surrounding tissue.

(3) *Saponine.*—This reagent, injected in certain concentrations together with spores, invariably leads to fatal infection in guinea-pigs. In mice, on the contrary, it fails to set up infection.

(4) *Calcium Chloride.*—At the suggestion of Capt. W. E. Bullock, R.A.M.C., attempts were made to study the influence of $CaCl_2$ as an infection initiator in tetanus. Notwithstanding the fact that $CaCl_2$ leads to a much less obvious disturbance than does, for example, trimethylamine, nevertheless it invariably induces the development of *B. tetani* when injected along with spores.

These observations indicate that the precise quality of the local "debility" at the nidus of infection is a factor of great importance in the development of *B. tetani* in the tissues.

(*e*) Experiments were, therefore, set up to determine the relative importance of the degree of tissue debilitation on the one hand, and of the number of spores inoculated on the other, as factors in the causation of tetanus.

This inquiry showed definitely that, in experimental animals at least, the degree of tissue debilitation is of much greater significance than is the number of spores inoculated.

Some of these experiments were extremely interesting. For instance, when an insufficient degree of local disturbance was produced, typical local tetanus, due to infection, was observed in certain of the animals (guinea-pigs were used in this series of observations). From this they recovered. Similar cases of local tetanus in experimental animals were also noted, even when the degree of tissue debilitation was relatively great, provided that the animals had been passively immunised with A.T.S. before or soon after inoculation with the "spore debilitant" mixture.

(*f*) The importance of the symbiotic factor—influence of the products of *B. Welchii*, etc.—in the initiation of tetanus infection naturally led to the question as to whether the infective capacity, or the toxogenicity, might not be reduced by the presence of certain organisms, just as it was enhanced by that of others. This was found actually to be the case. Certain mixed cultures obtained from wounds proved to be non-toxic, although they contained organisms having the morphological characters of *B. tetani* and which agglutinated with the Type sera. That these organisms were not deficient in toxogenic capacity was shown by injecting washed spores obtained from the cultures into animals, along with a tissue debilitant. When this was done, tetanus developed. Further, by growing one of the stock toxic cultures of *B. tetani* in presence of these mixed growths, the toxogenic capacity of the stock culture was greatly reduced. It has not yet been determined what particular organism or combination of organisms leads to this depression of pathogenicity. Neither can it be stated whether it is the mechanism of *infectivity* or that of *toxogenicity* which is thus depressed, although presumably it is the latter.

SECTION III.

Investigation of Serum Prophylaxis in Experimental Animals from the Standpoint of Infection.

The observations dealt with in Section II indicated a method of procedure whereby research could be applied to investigate immunity to *infection* which might be conferred by the administration of anti-toxin.

The inquiry brought out the following facts:—

(1) In experimental animals—guinea-pigs, rats, and mice—the prophylactic administration of anti-toxin failed to prevent the development of infection if the tissue debilitation at the site of inoculation exceeded a certain degree. This was the case even if the dose of anti-toxin corresponded weight for weight to as much as 40,000 units for a man of 60 kilos.

(2) Using guinea-pigs, it was found, that by increasing the unitage of anti-toxin administered prophylactically, the incubation period of the infection could be lengthened and the severity of the disease lessened. The quantities of anti-toxin used were 10, 20 and 30 units for animals of 250 grm. weight, giving a weight for weight ratio for man of 2400, 4800, and 7200 units. Saponine was the debilitant used, as it could be easily standardised by physical methods.

SECTION IV.

Inquiry as to whether—

(a) *Monotypical Anti-toxin exhibits Specific Anti-infective Properties.*

(b) *Inoculations of Cultures of* B. tetani *give rise to Anti-bacterial Bodies Specific to the Type of Organism used.*

(*a*) From this investigation it appeared that a monotypical anti-toxin was equally active in neutralising the toxin of any or all types of *B. tetani.* Some experiments suggested, on the contrary, that monotypical anti-toxic sera may, under certain circumstances, exhibit *anti-infective* properties in relation to the Types. The evidence of such relationship is so far only suggestive, and the statement is made with reserve.

(*b*) Experiments were then undertaken to determine whether any improvement in prophylaxis might be looked for from the employment of *anti-bacterial* sera. This investigation is still proceeding, but the following points appear to be definitely settled from the work already completed.

(1) *Anti-bacterial* sera, the anti-toxin content of which is negligible or nil, do not prevent infection.

(2) Evidence is so far lacking that *anti-bacterial* sera afford more adequate protection against infection than do pure *anti-toxic* sera of the same given anti-toxin content.

(3) The work done suggests that monotypical *anti-bacterial* sera may exhibit specific *anti-infective* properties in relation to Type. The results also suggest that this specificity is more marked in the case of *anti-bacterial* sera than in the case of *anti-toxic* sera. The results are, however, equivocal, and this question must remain undecided until anti-bacterial sera are available in larger quantities than is possible when laboratory animals are used for serum production.

(4) Experiments *in vitro* showed definitely that specific anti-bacterial immune bodies, other than agglutinins, could be produced by the inoculation of *whole cultures* of *B. tetani.* For technical reasons the anti-body studied was the "opsonin." The following facts were noted:—

(*a*) The opsonic activity of anti-toxic sera in the presence of *whole culture* is but slight.

(*b*) The opsonic activity of sera prepared by inoculation of *washed cultures* is slight, though the agglutinin titre may be high.

(*c*) The opsonic activity of sera prepared by the inoculation of *whole culture* is marked, and, furthermore, it is specific; but group relationship between the Types was noted in making these opsonic tests.*

(*d*) From the opsonic tests, it appeared that unfiltered cultures of *B. tetani* contain at least 3 antigens:—

(1) *Spasm-producing toxin,* which is filtrable, relatively stable, and non-specific.

(2) *Bacillary substance,* which is definitely specific to the Type, and stimulates production of agglutinins.

(3) *An anti-phagocytic substance* (? leucotoxin), relatively specific to each Type. This antigen is either unstable or not filtrable.

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* The technique used in carrying out these tests was the dilution method of Neufeld.

ADDENDUM.

An Inquiry into Surgical Procedures in Relation to Degree and Persistence of Anaërobic Infections of Wounds.

This inquiry, which was carried out by Miss Cayley, indicated that—

(*a*) No one of the antiseptics under investigation could be recommended as being of special value in the elimination of anaërobic infection.

(*b*) Anaërobic bacilli, even those of pathogenic significance, may persist in wounds until the completion of the process of repair.

(*c*) The degree of anaërobic infection of wounds that have been excised is, on the whole, less than in those that have not been so treated. Excision, then, while it does not eliminate infection, does so alter the condition of the wound that the harmful capacity of any anaërobes present is much reduced.

(*d*) In examining *in vitro* the influence of Brilliant Green and other aniline dyes on the growth of anaërobes, it was found that their activity was much reduced when cultures were made in Tarozzi tubes (4). This reduction of activity was more marked than that caused by the presence of serum. This observation has a direct bearing on the application of aniline antiseptics in surgical therapeutics.

Technique.

In making the investigation summarised in Section I, the following technique was elaborated :—

The swabs were taken from the deeper parts of the wounds as far as possible; then emulsified in 3 cub. centim. of sterile saline. Of this, 1 cub. centim. was inoculated into ordinary meat-water medium—Culture A. This culture gave an index of the non-sporing organisms present.

The remainder—2 cub. centim.—was heated to 80° C. for 10–15 minutes, or to 60° for 30–40 minutes. Of this, 1 cub. centim. was inoculated into a tube of meat-water medium and incubated anaërobically—Culture B. This gave an index of the sporing anaërobes present. The 1 cub. centim. which remained was inoculated into a tube of "selective" medium—designed to give an overwhelming growth of end-sporing bacilli—Culture C.

The "selective" enriching medium was prepared as follows :—

Take 1 lb. of chopped raw meat, add 1 litre of tap-water, and boil for 30 minutes in a water-bath. Cool to 45° C., make slightly alkaline to litmus, and add trypsin as for the preparation of Douglas' broth. Then incubate in an open vessel for four to five days at 37° C., allowing the material to undergo natural putrefaction. Filter the putrescent material so obtained through filter paper; neutralise to phenolphthalein at room-temperature. Prepare a conical sterile filter flask containing a layer of liquid paraffin, sufficient to cover completely the bottom of a flask to a depth of roughly 1/8 inch, together with 3 grm. of sodium formate, dissolved in 10 cub. centim. of distilled water per litre of medium.

The medium is then filtered, first through a Berkfeld and then through a Doulton candle, into the sterilised flask. The flask should be provided with a hooded delivery tube, so that the medium may be distributed as required. The medium can be kept in cold storage in the sterile flask, and keeps fairly well—about three weeks. It should never be sterilised by heat. Before use, the sterility of the medium should be tested by inoculating quantities from 5 cub. centim. to 0·1 cub. centim. into tubes of meat-water medium and incubated anaërobically for at least seven days.

If the medium is required for cultures that may ultimately be used for animal inoculation, it should be tested by the *in vivo* method, to ensure that the medium is in itself non-toxic.

Before use, fresh sterile kidney is added to the medium in the proportion of roughly 1/16 part of a kidney to 5 cub. centim. of the medium. The tubes to which the kidney has been added should be used within three days.

Prepared thus, the medium inhibits the growth of *B. sporogenes*, but allows of the growth of *B. tetani*, other atoxic round end-sporing, and certain oval end-sporing bacilli.

Latterly, in place of using meat-water tubes for cultures A and B, the following medium has been employed :—

Chop up finely the flesh of one rabbit, add 1 litre of tap-water and 5 grm. of sodium carbonate, and allow the mixture to decompose at 37° C. for roughly 16 hours. Then render the mixture slightly alkaline to litmus, and add 2 per cent. of trypsin. Incubate for a further period of 16–24 hours at 37° C. The material is then filtered through filter paper, rendered slightly acid, boiled to coagulate proteins, filtered again and neutralised.

Before neutralising at room-temperature, the samples of medium to be tested should be boiled and rapidly cooled.

The mean of two titrations with (1) phenophthalein and (2) α-naphthol-phthalein is taken, and the requisite amount of sodium hydrate added to the bulk of the medium.

Boil again and finally filter. Occasionally it may be necessary to pass the product through a Doulton candle to remove any dead organisms that might be present. The medium may then be tubed and autoclaved, or autoclaved in bulk and kept in cold storage. Before use, 1/16 part of a fresh sterile rabbit kidney should be added to each tube of 5 cub. centim.

Tubes which have been autoclaved and stored should be boiled for 30 minutes and rapidly cooled before the kidney is added, and should be used as soon as possible after the addition of the kidney. This medium gives very heavy growths of *B. tetani*, and appears to have some slight selective properties.

Manipulation Routine of the Cultures.

Tubes A, B and C were examined at 2-day intervals. When tube C showed a growth consisting mainly of end-sporing bacilli, the culture was filtered through a small piece of cotton wool to remove the kidney and sediment, and then centrifuged, emulsified in saline, and tested by the agglutination technique.

Not infrequently culture C failed to show growth, although round end-sporing bacilli might ultimately develop in tubes A or B. In such cases A or B were heated 60° C. for 45 minutes and sub-cultures made into the "selective" medium. In this way *B. tetani* was obtained from a number of cultures which failed to show any growth when inoculated directly from the swab into the "selective" medium.

The technique of the agglutination test and the preparation of agglutinating sera are fully described in previous publications dealing with the serological differentiation of *B. tetani* (1 and 2).

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The Existence of Daily Growth-rings in the Cell Wall of Cotton Hairs.

By W. Lawrence Balls, M.A., Sc.D. (Cantab.), late Fellow of St. John's College, Cambridge.

(From the Research Department, Fine Cotton Spinners' Association, Manchester.)

(Communicated by F. F. Blackman, F.R.S. Received February 25, 1919.)

[Plates 14–16.]

From studies of the growing cotton plant in Egypt* the author was led some years ago to the conclusions that the wall of the cotton-seed hair-cell was "probably composed of concentric layers, laid down during the active growth of each successive night, and numbering about twenty-five in all . . . they would thus, at the most, be about 0·0004 mm. in depth, so that their resolution by the microscope is highly improbable without some previous treatment."† Various methods were tried with the intention of bringing these layers into the limits of microscopic vision, but it was not until five years later that an accidental observation gave the clue to a method by which the limitations of microscope observation may be extended, and these layers made actually visible.

The observations which followed, demonstrating the existence of concentric layers in the wall of the cotton-hair as well as in the "fuzz-hairs," would have been interesting in any case on account of their bearing on all the physical and chemical problems which this typical cellulose presents. C. F. Cross has insisted on the necessity for considering cellulose problems in terms of "the ultimate fibre,"‡ but it now seems probable that the ultimate unit components must be the single layers composing the wall of the said fibre. The bare fact of the existence of such layers would have had no particular significance if it could not have been connected with previous precise study of the growth of cotton-hairs. By counting the number of layers in material previously preserved at known dates during the course of those studies, and remembering the cardinal fact that growth is daily arrested by the sunshine effect under Egyptian conditions,§ we have been able clearly to show that these layers are actually the growth-rings whose existence we had ventured to postulate. Knowledge of their real existence must materially affect some of our views concerning the physical properties of such hairs.*

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* Summarised in 'The Cotton Plant in Egypt,' London, 1912; 'The Development and Properties of Raw Cotton,' London, 1915, p. 79; "Analyses of Agricultural Yield," 'Phil. Trans.,' B, 1915–17, Parts I–III.

† 'Raw Cotton,' p. 79.

‡ Presidential Address by C. F. Cross, Society Dyers and Colourists, 1918.

§ "The Physiology of the Cotton Plant," 'Cairo Sci. Jour.,' July, 1910, *et. seq.*

Material.

The cotton material examined was chiefly of Egyptian origin, derived from my pure strain No. 77, on which most of the previous physiological work has been standardised, but samples from other Egyptian strains and varieties, together with cotton from other countries, have been used for check purposes.

In addition to mature cotton hairs (or "lint") and "fuzz," a small amount of material† pickled in acetic-absolute at all stages of growth had been brought by the writer from Egypt, amounting to 12 fruits in all. The growth-rings were shown by this equally well, in spite of five years and four months immersion in the 30 per cent. alcoholic solution of glacial acetic.

The seed-hairs of the cotton plant are of two kinds, the lint and the "fuzz." In certain wild cottons we find various groupings of abnormally long fuzz, short lint, or lack of differentiation between the two classes, which facts, in conjunction with the data from genetic studies of cultivated varieties, suggest that the two kinds of hair are intrinsically similar, in spite of the great difference between them in external appearance. In the case of pure strain No. 77, and most other Egyptian cottons, the fuzz-hairs are only about 1·5 mm. in length, clustered in two patches near the micropyle and base respectively of the seed, and exhibiting a vivid but unstable emerald green colour which fades to a rusty brown. Other cottons have fuzz which is devoid of colouring matter, while others again always show a brown colour without any precedent green. These colours occur also in the lint, a rare and little-known rogue strain of American (*G. hirsutum* ?) possessing vivid emerald green lint which quickly fades on exposure. The genetic peculiarities shown by various modifications, both of the seed-hairs proper, or lint, and of the subsidiary seed-hairs, or fuzz, are closely similar; this similarity extends not merely to colour-factors, but also to the distribution of each upon the seed-coat, and complex inheritance involving cryptomeres has been demonstrated in the latter respect for both by the writer.

The lint, or cotton fibre of common knowledge, is externally very unlike the fuzz, attaining a length of nearly 60 mm. in some varieties, and carrying a delicate brown colour at most, except in the green rogue above mentioned. The diameter of the embryonic fuzz-hair is nearly twice that of the lint-hair, but this difference is less obvious when both are mature; the convolutions, which are characteristic of the lint, are not so well shown by the fuzz, on account of its thicker cell wall, but they are present, and, in sum, we may reasonably anticipate that any phenomenon shown by the fuzz may be expected to be found, in some modified form, amongst the lint-hairs as well.

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* Harrison, W., "Investigations on Textile Fibres," 'Roy. Soc. Proc.,' A, vol. 94, p. 460 (1918).

† 'Raw Cotton,' p. 175.

Experimental Methods.

The first observation of these growth-rings was accidental, a laminated hair being noticed by the writer in some cotton treated by Cross and Bevan's method for the preparation of cellulose xanthate, with subsequent hydration.* The hair in question might well have originated from some source other than the cotton plant, but subsequent results have shown that it was a fuzz-hair. In any case, the phenomenon was so striking, and might prove to be so intimately related to the writer's previous researches in Egypt, that a systematic examination was undertaken, with the assistance of Dr. Mary Cunningham on the chemical side.

It was clear that the hydration process, or jelly formation, would need to be carried far enough to swell the wall of the cell to quite five times its initial thickness (fig. 1), and yet would have to be arrested some long way short of complete dissolution. In effecting this control we were able to take advantage of recent work by Cross and Bevan on the effects of CS_2 in conjunction with 9 per cent. NaOH (Engl. pat. 8342/18). At the best, however, we were unable to bring the process to complete certainty of demonstration in any one sample, so far as the growth-rings in the lint were concerned, though invariably successful with the fuzz. It seems evident that the attainment of the precise step at which the former are sufficiently swollen, and yet not too much, must be a matter of such careful chemical adjustment that the individuality of each hair may be concerned, and hence it must remain a matter of chance to a notable extent.† (Figs. 1, 6, and 7.) The point needs emphasis, for the sake of other workers.

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* Cross and Bevan, 'Researches on Cellulose,' 1905–18 (Pat. 8700/92, etc.).

† [*Note added in Press, April* 28, 1919.—After various trials Dr. Cunningham has obtained preparations with cuprammonium (Schweizer's reagent) which show these rings as clearly as any made with CS_2 and NaOH, for occasional hairs only. It is of further interest that some of these preparations were made from cotton cellulose, deprived of cuticle, *e.g.*, fully bleached and subsequently boiled in solution of alkaline sodium sulphite. By comparing various hairs in these preparations it seems clear that cuprammonium usually contorts the growth-ring strata too much. Thus, fig. 63, in Mr. Matthews' book on 'Textile Fibres,' undoubtedly represents growth-rings thus deformed, as usual, beyond obvious recognition as such. I am indebted to Dr. Coward for bringing to my notice another example, though a more dubious one, of unconscious observation of these structures. W. Minajeff ('Ueber das erhöhte Anfarben de rmercerisierten Baumwolle und dessen Ursachen," in 'Zeitschrift für Farben-Industrie,' vol. 15, p. 234 (1907),

Both for lint and fuzz we finally settled on the following treatment:—a preliminary boil in 1 per cent. NaOH, followed by acidification with 1 per cent. acetic and washing; then, evacuation of the receiver with a Geryk pump, and injection with 9 per cent. NaOH *in vacuo;* some 3 c.c. of the soda having been used for 0·2 grm. of cotton, the receiver was opened, 3 c.c. of CS_2 added, and allowed to stand at room temperature. Samples which would swell up on wetting could usually be withdrawn within half an hour, although the reaction does not reach equilibrium until some hours have elapsed. After three or four days the hairs begin to revert, and the growth-rings are largely obliterated in the process.

Thus far we have not been able to make permanent microscope preparations, though slides mounted in water will keep for two or three days, and even show the rings in the lint upon being wetted after they have dried up.

The microscope objectives used were an old Swift's 1/3-inch and 1/6-inch, supplemented by Watson's Versalic 1/12-inch oil immersion. Fuzz rings can be recognised clearly with the first of these, and the presence of lint rings can be suspected by an indefinable appearance. Most of the phenomena described can be seen with the 1/6-inch, which gave a magnification of 250 diameters on the bench with the eyepiece used, while the 1/12-inch was chiefly employed to corroborate in counting the rings.

The illumination found most satisfactory for the lint rings was obtained from a nitrogen-filled lamp at a distance of 2 metres from the microscope, using the concave mirror and not employing the condenser substage; the substage diaphragm was kept wide open. The line drawings (figs. 8–11) were made with assistance from arc lamp projection and a prism, while for the fuzz photographs I am indebted to Messrs. Flatters and Garnett. The lint photographs were made by the writer with an extemporised apparatus. Most of the microscope equipment, owing to the impossibility of obtaining new apparatus at the time, hardly seemed adequate to the delicacy of the cytological problem.

The final magnifications obtained depended on the amount of swelling undergone by the wall, and in extreme cases the successive layers may be magnified as much as 20,000 times their estimated original thickness. This was made up of a forty-fold swelling and 500 diameters magnification with a 1/12-inch lens. The usual magnification, however, begins with a swelling of

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figures four and five layers only in several adult hairs which had been swollen by cuprammonium. These appearances are described as fissures ("Ritzen") in the cellulose. While neither the description nor the drawings fit real growth-rings, it is possible that Minajeff may actually have seen them, but, not realising their nature, only noticed four or five out of two dozen.]

of five to tenfold, and with this amount there would seem to be no serious destruction of the cellulose structure.

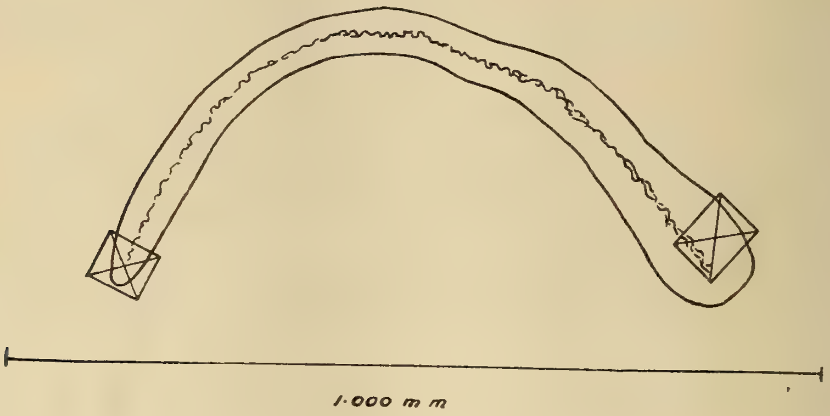

Fig. 8.

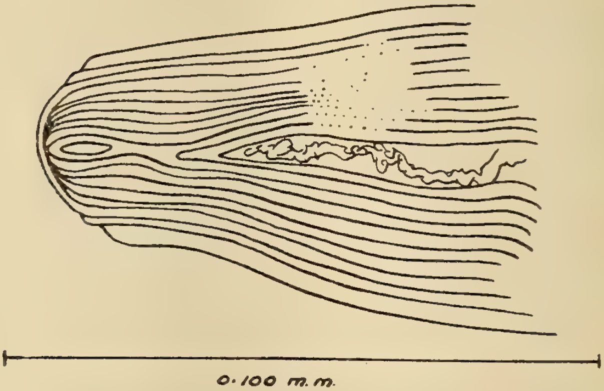

Fig. 9.

Figs. 8 and 9.—Complete fuzz-hair swollen by treatment, and details of growth-rings in marked area at its tip. The two outermost rings broken away over actual tip. The isolated portion of the 10th ring could not be seen to be in connection with the main bulk of the ring.

Observations.

Intelligent understanding of organic structure must depend on knowledge of its growth-history, and it will therefore be more convenient to treat the observations from this point of view.

The differentiation of epidermal cells on the testa of the cotton-seed to form lint-hairs up to a length of 1 or 2 mm. was described by the writer in 1905.* Certain manipulative difficulties intervened to prevent further

* "The Sexuality of Cotton," 'Khedivial Agric. Soc. Year Book,' 1905.

study of the developing fruit, and no method was found until 1913, when the story was completely outlined* in connection with the physiological studies

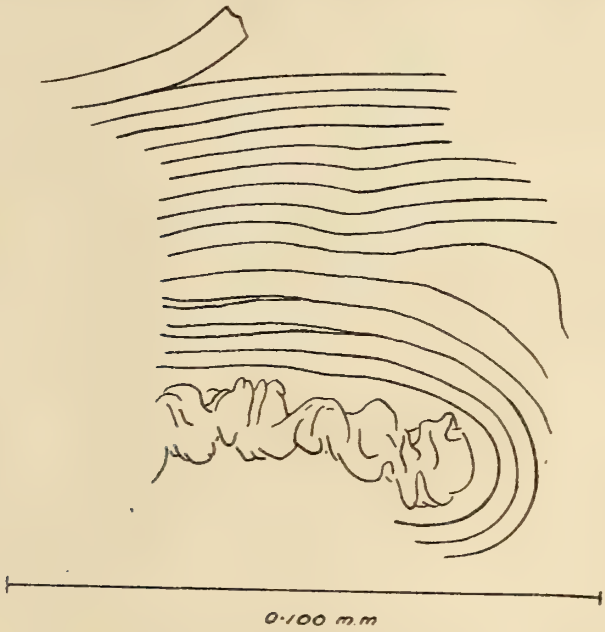

Fig. 10.—As preceding, but showing portion near base.

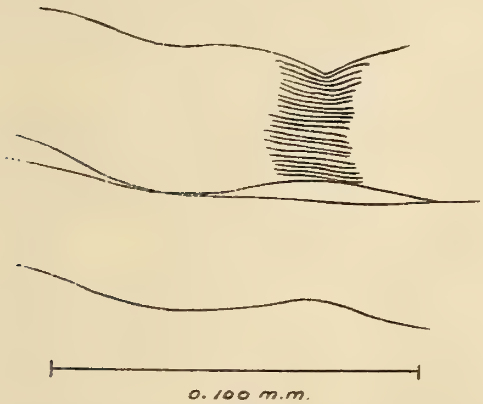

Fig. 11.—Adult lint-hair, showing swollen dimensions, and 23 rings as observed and repeatedly counted. Original diameter of cell lumen practically unchanged, *i.e.*, approximately 0·010 mm. on major diameter, with wall-thickness of 0·004 mm., making up a "ribbon-width" of 0·018 mm.

The wall-thickness of 0·004 mm. has been swollen to 0·030 mm.

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* 'Raw Cotton,' pp. 80–85, etc.

of the process. The results were in no way peculiar on the cytological side, the hairs being simple cells with a large single nucleus and persistent cuticle, which grow to their full length during the first half of the maturation period of the fruit, and lay down secondary deposits of cellulose on their primary cuticularised wall during the latter half of the maturation period. For the purpose of these studies a large number of open flowers of my pure strain No. 77 were labelled on July 9, 1913, at the Giza Cotton Experiment Station, and a few were collected and pickled every three days until the fruits opened around the 50th day; a single boll from several stages was available for the present observations, of which the following are representative.

Nine-day Fruit.—The lint and fuzz are scarcely altered by the hydration process, and it is debatable whether any swelling of the wall has taken place at all. Judging by the proximity of the protoplasm to the cuticle at points where a hair had been accidentally bent, this swelling, if any, does not increase the wall thickness to more than 8 per cent. of the cell diameter, under parallel treatment with the well-swollen later fruits. Judging by the slight increase in visibility of the cuticle, such swelling may have happened, and this is supported by indications of wrinkling of the cuticle in surface view.

The primary cell wall can only contain a relatively small amount of cellulose, and probably the cuticle constitutes not less than one-third of its weight.

Twenty-one-day Fruit (fig. 2).—In spite of the great increase in lint length, which now approaches its adult value, the same description applies as for the nine-day specimen, with the exception of perhaps one hair in every few hundred, which is slightly swollen, so that the cell diameter is increased by about 30 per cent.

Twenty-seven-day Fruit (fig. 3).—Previous work had shown that the secondary thickening, with its concurrent formation of simple pits in the wall,* and the consequent ability of the hair to twist on drying, began round about the 21st–25th day.

Conformably with this we find that these hairs, under the xanthate treatment, present the typical beaded appearance. The great majority are swollen to three or four times the cell diameter, and the wall thickness in the swollen state may be equal to the cell diameter of the untreated hair. The remains of the cuticle, torn by this swelling but not themselves swollen, assume the position of girdles of various widths, or of spiral bands; both these formations are familiar as the result of treatment with cuprammonium solvent.†

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* 'The Cotton Plant in Egypt,' p. 84, and 'Raw Cotton,' pp. 74, 77.

† O'Neill, C., 'Calico Printing and Dyeing,' London, 1862, and others.

At this stage, in hairs which had not swollen to the extent described above, very fine transverse wrinkling of the cuticle was seen, indicating that the transverse swelling of the secondary wall is accompanied by longitudinal contraction. This fact, which is indeed a matter of common experience with other reagents such as NaOH, propounds a question as to the space-dimensional internal structure of the cellulose wall, which has not been adequately answered.

The contraction is also demonstrated by the form which the remains of the protoplasm assume, since measurements show that this part of the hair is not appreciably swollen by the treatment, and yet it is evidently put under longitudinal compression. It follows that the cellulose wall in every degree of hydration, and presumbly, therefore, in its original untreated state, has some internal construction which is not the same in a tangential direction as is the case radially.

The swollen wall found in the great majority of the hairs at this stage is sometimes homogeneous, within the limits of microscopy, and sometimes shows a denser zone whereby the wall is divided into two portions. The outer of these two is the wider. No cases of more than two such portions have been noticed in the material examined, though it is probable that one or two more might be found exceptionally. Assuming that these layers might well be due to the growth of successive nights, and be demarcated from each other at the daily growth stoppage brought about by the sunshine effect, we may state that the average number of growth-rings at this stage is about 1·0; some hairs have two, while some have none. A variation of two days, plus or minus, in the dating of these events in the life history of the fruit is quite likely at this stage; the full maturation of the fruit in this strain has a P.E. of $\pm 3{\cdot}0$ per cent. which allows an extreme chance variation of $2\frac{1}{2}$ days either way at this present stage; in other words, the number of growth-rings may be expected to vary over a range of five, even if the individual hair-cells are no more variable than the massive tissue structure of the fruit.

Thirty-day Fruit.—The appearance of the fuzz-hairs at this stage is most strikingly different from that of their younger predecessors. The occasional dense layer seen in the swollen wall at 27 days is now multiplied, and these layers have become the most conspicuous part of the object, on account of their smooth curves. The first growth-ring is still quite twice the thickness of its successors; on tracing it to places where it passes under a girdle of cuticle there seems reason to believe that this extra thickness is not due to less constraint on its swelling, but that it was initially a thicker deposit of cellulose than its successors.

The lint-hairs also show lamination at this stage for the first time, but none have been observed on which dependable counts could be made.

Upon counting the number of growth-rings in the wall of 24 fuzz-hairs, the following frequency was obtained:—

Rings	0	1	2	3	4	5	6	7		
Number of hairs	0	2	3	8	5	3	1	2	= 24.	Mean = 3·6

These figures are too small for any statistical generalisation, but it is evident that they vary only a little more than we anticipated, and that the mean is approximately three rings higher as the result of three days more growth.

Thirty-three-day Fruit (fig. 4).—The general appearance of this stage is like that of the previous one, but the count of growth-rings in the fuzz gave a mean value of 5·3, being 2·7 higher than the fruit of three days previously, with the following frequency distribution:—

Rings	0	1	2	3	4	5	6	7	8	9	10	11
Number of hairs	—	—	—	4	4	3	3	—	2	5	1	1

Photographs of one lint-hair showing ten rings, are reproduced in figs. 6 and 7, Plate 16.

Thirty-six-day Fruit (fig. 5).—The increase in the number of rings in this slide merely brought the value up to 6·6 on the first count taken, for reasons which will shortly be described. The frequency distribution was:—

Rings	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Number of hairs	0	0	0	1	1	4	1	4	5	5	3	2	0	0	0	0

The scatter of the observations is much the same as before, since 3 per cent. P.E. would allow seven days extreme variation at this age, against nine days actually observed, but the next two stages show that an observational error is creeping in.

Meanwhile we may note that four lint-hairs in this stage were in a state which enabled reliable counts of their growth-rings to be made, and were found to contain 7, 10, 11, and 12 rings respectively.

Thirty-nine-day Fruit.—The counts of the fuzz-rings were substantially identical with those from the previous stage, but the preparation was better than the previous one as regards the lint, and seven hairs showed 10, 12, 13, 13, 13, 14, and 14 rings respectively. Subtracting the mean value, 13 rings, from 39 days, brings us back correctly to the 26th day as the one on which the first ring was formed.

Forty-two-day Fruit.—In this preparation the mean apparent number of fuzz-rings was again unchanged (7·2), though one very definite example of 14 rings was noted. No countable lint rings were found.

Mature, or Fifty-day Fruit.—The ripe fuzz and lint were examined in the pickled material, but also in various samples of lint and of seed, both of this No. 77 strain, and of others grown in Egypt and the Sudan. Less regular rings were found in some American Upland and Indian lints. The counts of rings in the lint could never be proved to exceed 25 in number, and the smallest figure obtained with certainty was 20, these numbers agreeing completely with expectation. Not more than 16 obvious rings have yet been seen in the adult fuzz, but it was presently found that the very decided line of demarcation, which the older growth-rings of the fuzz display, is prone to become less definite in the later, innermost rings. Further search showed that in some cases this decrease in definition was progressive after the first three or four rings, while in others it was quite sudden, and occasionally it was possible, in lucky preparations, to see clearly that the innermost "ring"—as casual observation had judged it to be—was actually compound (fig. 5 shows traces) and actually consisted of seven or even more rings which were indistinguishable from those shown by the lint.

This accounts for the apparent deviation of the number of rings in the fuzz from expectation based on our hypothesis. It does not exclude the possibility, in all the other cases where the evidence was negative, that the fuzz ceases to thicken its wall at an earlier date than does the lint, but the trend of the facts is evidently opposed to the latter assumption, and it is more reasonable to assume that the growth of secondary thickening in the fuzz is less "inhibited" at first than in the lint. The converse inhibition would seem to obtain as regards growth in length.

It is a striking fact, well in line with all the data from genetics and from the ordinary cytology of development, that the fuzz-hairs should degrade to the production of walls resembling those of the lint-hairs, when they are in a senescent condition,* and not before. The problems of cell-senescence in the cotton plant would seem to provide a most promising field for research.

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* "Temperature and Growth," 'Ann. Bot.,' p. 557 (1908); 'Raw Cotton,' pp. 44, 96, 99, etc.

Anomalous Secondary Thickening of the Cell Wall.

While engaged in counting these rings in pickled material of known age, an abnormality was encountered under circumstances which indicate that two layers of cell wall may be formed simultaneously inside one and the same cell. Our observations thus link up with those of other workers on the sculpturing of spores on one hand, and with straightforward cell-physiology on the other.

It is not unusual to find in preparations made by the present method that actual lacunæ occur in the thickness of the wall, due to the cellulose of some one night's growth not having been "bonded" firmly to that of the previous morning. It is not difficult to imagine that this might result from various minute accidental causes.

In one hair, however, the writer observed a phenomenon for which he has found no exact parallel in cytological literature, though it suggests comparison with the behaviour of plasmolysed cells, and may have some relation to the debated subject of the growth of the sculptured exine on the surface of pollen-grains.

This hair was noticeable with a low magnification, having one very conspicuous growth-ring, seen in optical longitudinal section as a line in the wall on both sides, running from the base of the hair to a distance of 2 mm. along it. The whole of the hair could not be seen, but a length of 6 mm. was measured, and in all respects—except this conspicuous ring—it was a normal lint-hair. With higher magnifications it was found to be in such condition of hydration near its base that ordinary growth-rings could not only be seen, but counted, in several places. Most unfortunately, however, this state of preparation did not extend more than 1·5 mm. along the hair, so that no rings could be counted at the point where the conspicuous one, which had been obvious under the low power, died away. This conspicuous one appeared to be a surface of actual cleavage, though a recognisable lacuna was nowhere clearly visible, and at first it appeared to be merely an extreme case of the lacunæ already mentioned.

On counting the growth-rings, inside and outside this surface of cleavage, which we were able to effect in three separate places, the number of rings outside the cleavage was eight, while inside it there were either 13 or 14. The material in which this hair was found, came from a 42-day fruit; we have seen that the expected number of growth-rings at this stage is only 16, whereas we found 21 or 22. The highest number found in the 39-day fruit was 14, which in three days more becomes 17. The excess number of rings in this abnormal hair, above expectation, is almost in excess of the limit of expectation. Also we have found that scarcely any hairs had begun to form their first rings on the 21st day, so we have either to assume that this hair was extremely early, as well as being abnormal otherwise, or else that we have here a case of dual wall-formation for the first eight days.

The writer personally inclines to the latter opinion, though the probabilities can just, and only just, be strained to bring the case into category of normal wall-formation; it seems therefore desirable to put this interesting observation on record.

Conclusions.

The following facts appear to be firmly established as the result of combining these observations with antecedent studies:—that the primary wall of the seed-hairs in cotton contains very small amounts of cellulose; that the secondary thickening of the wall proceeds intermittently under normal Egyptian field crop conditions, being arrested each afternoon; that the cellulose of the hair consequently consists of a number of concentric shells, layers, or "growth-rings," each one representing one day's growth, with the exception of that of the primary wall; and that the so-called fuzz-hairs are analogous with the lint-hairs, though their growth-rings are coarser and more sharply demarcated. The hairs are covered, outside the cellulose of the primary wall, by a cuticle, bearing wax, which is structurally and historically identical with the cuticle of the testa, while it is structurally and chemically distinct from the cellulose. The secondary wall, but not the primary, is traversed obliquely to the hair axis by simple pits which are rarely visible except in the living hair, and to these pits is due the twisting of the hair and its characteristic convolutions after death.

In the method employed we now possess a simple kind of ultra-microscopy, applicable to cellulose. The dimensions of the wall of the lint-hair are such that the thickness of each of the 25 growth-rings composing it can only be, at most, about 0·4 μ, much less than the wave-length of sodium light.

Summary.

The present communication describes the structure of the cellulose wall of the cotton hair, in relation to its development, as a continuation of observations previously published in "The Development and Properties of Raw Cotton."

By suitably swelling the cellulose-wall to some five or ten times its initial thickness, under treatments with CS_2 and NaOH, concentric layering becomes visible in the swollen walls.

Material of known age and development, fixed in Egypt, was examined by this method and it was found that there is only one thin primary layer while the hair is growing in length, but, that as soon as thickening of the wall sets

in, the number of layers increases day by day up to a maximum of 25, when thickening is complete.

As growth is arrested by sunshine in the middle of each day in Egypt, the number of these layers corresponded to the number of nights during which the growth in thickness of the cell-wall had continued.

These concentric layers in the wall of Egyptian cotton-hairs are thus rings of nightly growth, differentiated by heterogeneity like the annual rings in timber.

These rings are coarser, more sharply demarcated, and less regular, in fuzz-hairs than in lint-hairs. The later rings in fuzz-hairs may resemble those of lint-hairs.

Certain abnormal appearances indicate that the cellulose wall may grow for a time without being in full contact with the ectoplasm.

I have to acknowledge much valuable assistance in our laboratory from Dr. Mary Cunningham in the adjustment, application and interpretation of the chemical treatment, and from Mr. C. F. Cross, F.R.S., in directing my attention to the chemical problems of cotton.

My thanks are also due to the Fine Cotton Spinners' and Doublers' Association, Ltd., of Manchester, for permission to publish this note, and for having made possible the continuation, extension and application of my previous researches.

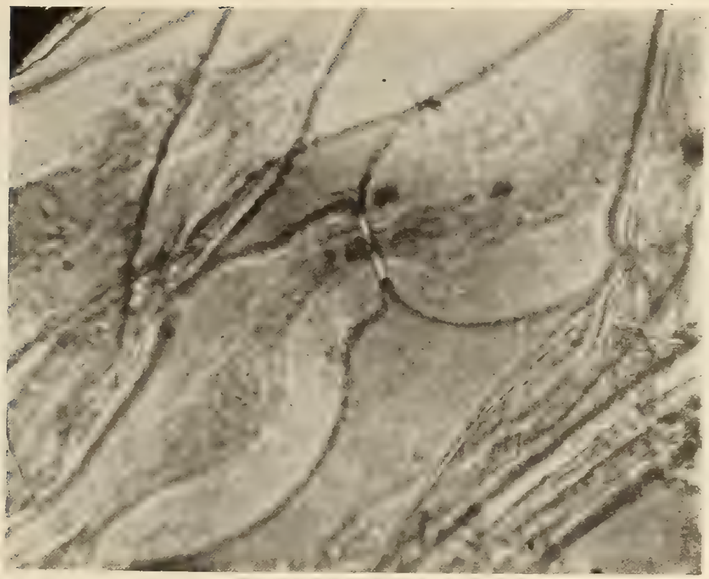

Fig. 1.

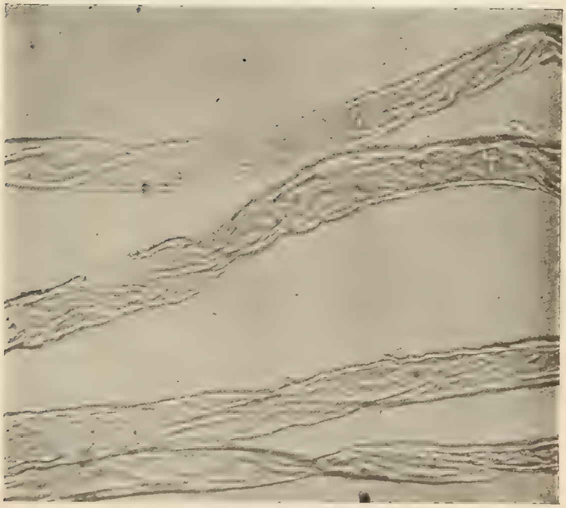

Fig. 2.

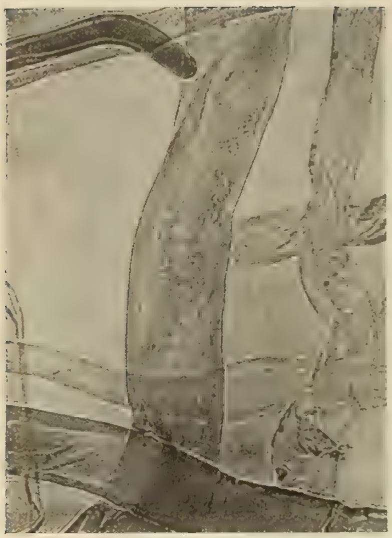

Fig. 3.

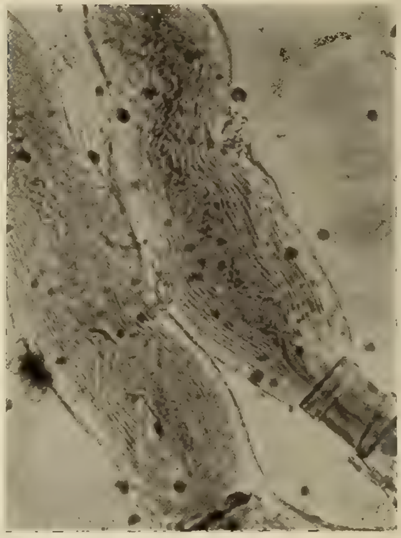

Fig. 4.

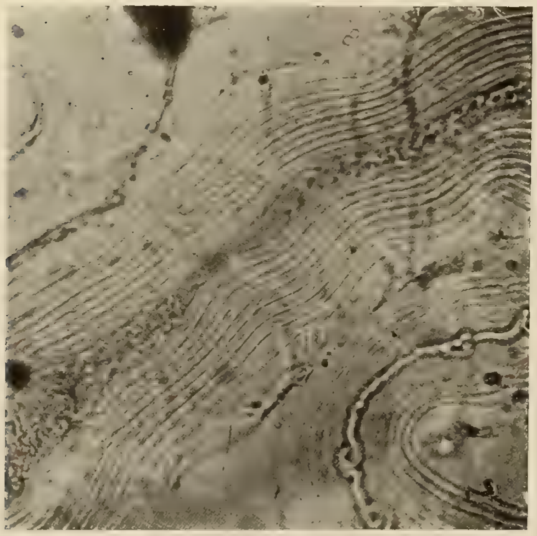

Fig. 5.

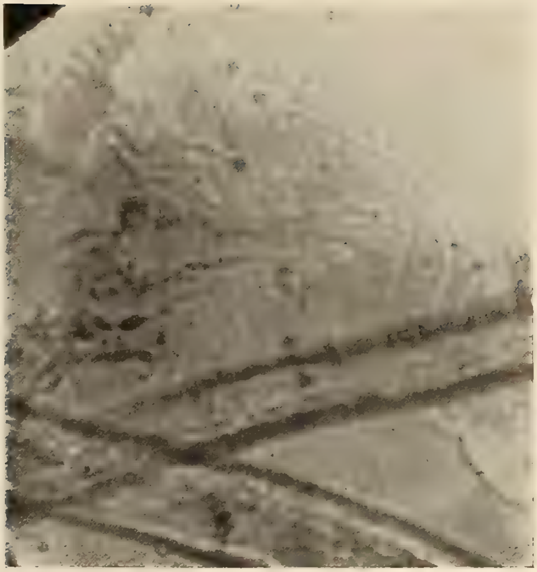

Fig. 6.

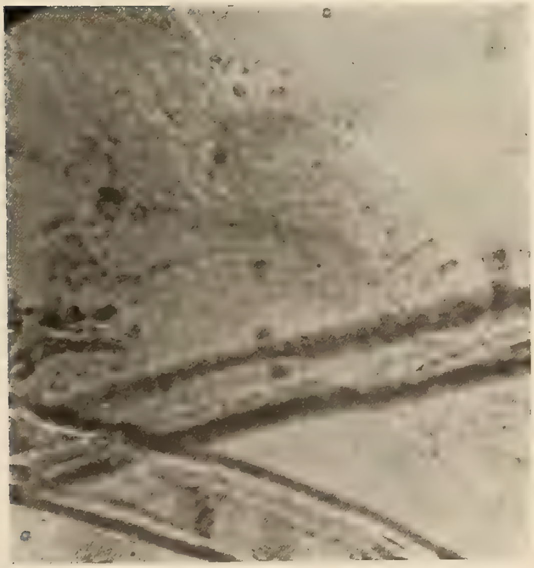

Fig. 7.

DESCRIPTION OF PLATES.

(All figures represent hairs of the author's No. 77 Pure Strain of Egyptian Cotton. Acetic-absolute pickle, five years old.)

Plate 14.

Fig. 1.—Lint-hairs swollen to varying extents under nominally uniform treatment by CS_2 and NaOH. A cuticle girdle clearly shown, indicating original diameter of swollen hair. × 540.

Fig. 2.—Fuzz-hairs on 21st day of development of fruit (boll). Primary wall scarcely swollen by treatment, wrinkled and flaccid as in all preceding 20 days. × 360.

Plate 15.

Fig. 3.—Fuzz-hairs on 27th day, secondary thickening of wall having begun about 26th day. Two hairs well swollen, with only one growth-ring visible ; demarcation of secondary deposit from primary wall is indicated. × 360.

Fig. 4.—Fuzz-hairs on 33rd day. Fine example of a wide girdle of cuticle ; also faint spiral of same. Nine and ten growth-rings ; constriction in girdle clearly shown. Swelling of cell-diameter is about three-fold, of wall is about seven-fold. × 360.

Fig. 5.—Fuzz-hairs on 36th day. Cuticular remains very evident as spirals. Firmness of growth-layers in the jelly under bending stresses can be seen at corners of photograph.

Central hair happens to have same number of growth-rings as one in fig. 4 ; the great difference in appearance is thus merely due to the greater swelling, which has magnified the cell-wall at least fifteen-fold. Magnification of growth-rings in this photograph is thus $15 \times 360 = 5000$ about.

Plate 16.

Figs. 6 and 7.—Lint-hairs of 33rd day (for fuzz see fig. 4). Two successive photographs with minute alteration of focus ; original focussing effected directly on surface of a plate, not on ground glass. The two hairs practically unaffected by reagents indicate original dimensions of cell-wall. The swollen hair girdled by cuticular spirals passed into complete solution a little way outside the field of view ; maximum swelling in the field is about seventeen times the wall thickness. It is difficult to photograph the hyaline layers satisfactorily in optical section, but in various places the ten rings are indicated as a series of shadings. × 540.

Maximum magnification of growth-rings here is $17 \times 540 = 9000$ about.

Croonian Lecture.—*The Biological Significance of Anaphylaxis.*

By Dr. H. H. Dale, F.R.S.

(Lecture delivered May 29, 1919.)

(Abstract.)

Anaphylaxis was regarded by Richet, who first clearly recognised the phenomenon, as the opposite of immunity or "phylaxis." At an interval of some weeks, after a first dose of any one of a group of poisonous proteins, the animal was found to be apparently much more susceptible to the action of the poison in question. Further investigation has shown that this susceptibility is not connected with the naturally poisonous properties of the substance used, but can be developed in relation to perfectly harmless protein substances, provided they are obtained from a different species and introduced into the system without hydrolytic cleavage. The sensitiveness is highly specific. It discriminates between corresponding substances from different species, between materials from different organs from the same species, and between individual proteins from the same organ. It can be transferred to a normal animal by blood or serum from an anaphylactic animal. In the nature of the substances producing it, in the limits of its specificity, and in the possibility of its transfer by serum from a treated animal, it shows a very suggestive correspondence with the type of immunity associated with "precipitin" formation. A highly precipitating serum from an immunised animal confers anaphylaxis on a normal animal more readily, *i.e.*, in smaller dose, than serum from an animal itself anaphylactic. Nevertheless, the serum from an anaphylactic animal forms no visible precipitate with the antigen, and an animal whose serum has this obvious precipitating quality is not anaphylactic, but immune. Anaphylaxis is not so much the direct opposite of immunity as an anomalous concomitant of a certain phase in its development. An animal rendered anaphylactic to a naturally poisonous protein is immune to the natural poisonous action, but has acquired a new sensitiveness to it as a protein.

The symptoms following injection of the sensitising antigen into an anaphylactic animal are characteristic, not of the substance but of the species exhibiting the reaction. In the guinea-pig the most conspicuous feature is an intense tonus of the plain muscle, which by causing a valve-like closure of the bronchioles produces rapid asphyxiation. In the dog the central feature is a poisoning of the endothelial wall of the capillary blood vessels, especially in the liver, causing a shock-like collapse of the blood-pressure and hæmorrhages into mucous membranes. In the rabbit the heart muscle seems to be primarily affected. They resemble closely the characteristic types of action, on these same species, of a large class of naturally poisonous proteins, protein cleavage products, and organ extracts, and of the organic base amino-ethyl-glyoxaline (histamine). Blood in clotting acquires toxic properties of a similar type. The complex can be analysed into an action mainly on two tissues—stimulation of plain muscle and poisoning of the vascular endothelium. These two effects appear with different relative prominence in the different species.

Several theories have been put forward to explain the anaphylactic phenomena. It is agreed by all that anaphylaxis is due to the presence of a specific antibody of the precipitin type.

1. It is supposed that the formation of the complex of antigen and antibody in the blood leads to a rapid digestive hydrolysis, with the liberation of poisonous cleavage products.

2. It is supposed that the union of antigen and antibody produces a disturbance in the equilibrium of the blood colloids, initiating pre-coagulation changes and rendering the blood toxic to the tissues.

3. It is supposed that the difference between anaphylaxis and immunity is due to the different distribution of antibody between the cells of the tissues and the blood-plasma. The occurrence of the reaction between antigen and antibody in the tissue cells is regarded as the cause of the anaphylactic symptoms. Evidence in favour of the last hypothesis is afforded by the reaction of isolated plain muscle from (actively or passively) anaphylactic and immune guinea-pigs.

The meaning of the "specificity" of anaphylaxis and immunity is discussed, in the light of the recent work by Dakin and Dudley, which gives the first hint of the difference in molecular pattern between corresponding proteins from different species.

There has been a general tendency to interpret the anaphylactic reaction in terms of the action of poisonous cleavage products of proteins, explaining the symptoms by assuming the formation of the products having this type of action. It is suggested that the true order of interpretation may be in the inverse direction; that more is known of the nature of the change in the cells which results in the anaphylactic shock than of the mode of action of the substances which produce analogous symptoms in the normal animal.

Further study of the anaphylactic reaction may throw light on the action of naturally poisonous protein-derivatives and drugs, and on the intimate physiology of plain muscle fibres and other cells.

OBITUARY NOTICES

OF

FELLOWS DECEASED.

CONTENTS.

Page

Sir William Henry Power i

Clement Reid (with portrait) viii

Daniel Oliver (with portrait) xi

Captain Geoffrey Watkins Smith xvi

Hermann Graf zu Solms-Laubach (with portrait) xix

Robert Bell xxvi

Edward Hull (with portrait) xxviii

Keith Lucas (with portrait) xxxi

Henry George Plimmer (with portrait) xliii

Alfred Merle Norman (with portrait) xlvi

Sir WILLIAM HENRY POWER (1842–1916).

Sir William Henry Power, K.C.B., Principal Medical Officer of the Local Government Board from 1900 to 1908, died on July 28, 1916, at his residence at East Molesey after a lingering illness. He was in his 74th year, having been born in London, December 15, 1842. His father, Dr. William Henry Power, who died in 1877, apart from his strictly professional work, had earned a remarkable reputation as a successful medical coach in the various subjects for the diplomas of the Royal College of Surgeons and of the Apothecaries Company. It is of interest to note that the following of medicine as a career was hereditary in the family to a somewhat unusual degree, Sir William Power being the fifth representative of the profession in direct succession from father to son, the first of the medical line having been John Power (born in 1730), who practised as a surgeon at Polesworth in Warwickshire.

As bearing on Sir William Power's devotion to exact methods of research it may be noted also that evidence of hereditary devotion to mathematical studies is afforded by the fact that his uncle, John Arthur Power, and at least four other relatives on the paternal side graduated at Cambridge as Wranglers and became Fellows of their respective Colleges; one of them, John Power (1818–1880), who was 8th Wrangler in 1841, eventually becoming Master of Pembroke College, Cambridge, and Vice-Chancellor of the University.

Power received his early education at University College School, subsequently commencing his medical career in the manner then usual by being apprenticed to his father, and entering as a medical student at St. Bartholomew's Hospital. He obtained the qualifications of M.R.C.S. and L.S.A. in 1864, and during the next six years held various hospital appointments, of which a somewhat prolonged tenure of that of Resident Medical Officer to the Victoria Park Hospital for Diseases of the Chest afforded him the opportunity for obtaining an intimate practical knowledge of the various clinical and pathological phases of tuberculosis, of which he availed himself to the utmost. He always retained special interest in the study of this disease, more especially in relationship to public health work, and the results of this early training, coupled with his intuitive appreciation of the various problems requiring solution and of the methods of scientific investigation best suited for their elucidation, proved most valuable, more especially during his term of service on the Royal Commission on Tuberculosis.

In 1871 Power commenced his long official career in Public Health on appointment as Temporary Medical Inspector to the Local Government Board, to which the Medical Staff of the Privy Council had been transferred on the formation, in that year, of the new Department. Of his colleagues at this period no less than four, John Simon, Seaton, Buchanan, and Thorne, as in the case of Power himself, subsequently became in turn Principal Medical Officer to the Local Government Board. Of this pioneer staff, which also included Netten Radcliffe and Ballard, Sir John Simon has pithily stated the aim and objects in the following words: "I believe we had the credit of earnestly endeavouring to learn the truth, and tell the truth, as to the matters which our enquiries regarded."

In the course of 16 years' service as Medical Inspector Power carried through an immense amount of more or less routine work, including enquiries into the sanitary circumstances and administration of various urban and rural districts. In addition he investigated and reported on a number of outbreaks of infectious diseases, more particularly small-pox, diphtheria, and scarlet fever, as to which some brief account must be given in view of the importance of the discoveries made, and of their bearing on epidemiology and preventive medicine.

For instance, while investigating the incidence of small-pox in the area surrounding the Fulham Hospital in 1881 and subsequently in 1884–1885, Power found that admission of cases of this disease into the hospital at certain periods was followed after regular intervals of time by the occurrence of cases of small-pox in the surrounding district. He demonstrated, moreover, the fact that if a circular area extending outwards from the hospital as a centre, to a distance of a mile, was divided into zones drawn upon the map having radii of $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$, and 1 mile respectively, and an enumeration of all the houses in each zone were made and also of all houses invaded by small-pox, the percentage of invaded houses in each zone diminished as the distance from the hospital increased and, further, that this relation held good in each quadrant of each zone. Within the quarter-mile zone there was but one approach to the hospital, this being in the north-west quadrant. This being so, the distribution of the cases showing exceptionally heavy incidence in the south-west quarter-mile quadrant was not such as to suggest any relationship to lines of traffic or ambulance routes. Studying the observed phenomena more closely, Power concluded that diffusion of small-pox only occurred subsequent to the aggregation of acute cases in the hospital, and probably also only during prevalence of certain atmospheric conditions, the effect of which, however, seemed to vary somewhat from one season to another.

Finally he directed attention to the fact that statistics of small-pox incidence in the registration districts of London during the years 1876 to 1885 afforded demonstration that the local distribution and intensity of small-pox during the epidemics which had occurred during that period had been definitely influenced by the proximity or otherwise of the London small-pox hospitals. He pointed out, moreover, that such relationship tended to become specially marked in the event of any new hospital situated in a district up to that time comparatively free from small-pox having to be opened for the reception of acute cases of the disease. This now classical exposition of the danger to the public health incidental to the aggregation of cases of small-pox formed the basis of legislative action which resulted in the removal of small-pox hospitals out of the Metropolitan area.

Power was the first to direct attention (in 1878) to the possibility of the dissemination of diphtheria from the consumption of milk. His report to the Local Government Board on an "Epidemic Prevalence of Diphtheria in North London," dated December, 1878, contains a lucid account of the line of reasoning which led him to the conclusion that milk was capable of acting as a medium for the conveyance of diphtheria infection to the human subject. Although the source of infection of the milk itself was not traced, demonstration in detail was afforded that the disease had been conveyed along with milk distributed by a particular dealer serving the affected area.

In 1882 Power, as the result of inquiry into an outbreak of scarlet fever in St. Giles and St. Pancras, demonstrated a similar relationship between the consumption of infected milk and the spread of scarlet fever: suggesting, moreover, the possibility that milk-borne scarlet fever might be due to a disease of similar character in the cow rather than to contamination of the milk from a human source.

His investigation of a sudden and extensive outbreak of this disease which occurred in Marylebone and certain other districts in December, 1885, carried the matter a stage further by the interesting and important discovery that cows suffering from a vesicular disease of the teats and udder constituted the actual source of infection.

Towards the end of the nineteenth century it became apparent that the water supplied for drinking purposes in various areas, in Yorkshire particularly, was responsible for the causation of more or less extensive outbreaks of lead-poisoning. The occurrence of a number of deaths among affected individuals naturally directed attention to the special circumstances, and it shortly became obvious that, in every instance, it was specially soft water of acid reaction, derived from moorland gathering grounds, and delivered under high pressure, that alone was capable of dissolving lead, sometimes in considerable amount, from the local service pipes.

So soon as information resulting from preliminary investigation of certain of these outbreaks became available, Power, who evinced much interest in the matter, at once realised that factors, in all probability hitherto overlooked, or non-existent, must have come into play. To him it is that we owe the original suggestion that the acidity to which it appeared that the plumbo-solvent action of soft moorland waters was due, was in turn dependent on the presence in the water of low forms of organic life. He further suggested that these might find their necessary pabulum in the peaty material abundantly produced on the gathering grounds from which such waters are derived. And it was at his instigation that to Dr. Houston, now Director of Water Examinations to the Metropolitan Water Board, was entrusted an experimental inquiry on the subject of acid-producing bacteria in these moorland waters, and, further, of the plumbo-solvent action of the acid produced by the bacteria in culture media. As an outcome of this and other investigations on the subject, of which one at least owed much to Power's valued suggestions and kindly criticism, simple methods have been devised for neutralising the action of any acids present in the water, with the result that outbreaks of lead-poisoning from drinking-water in this country are now practically unknown.

Reference must also be made to an aspect of Power's prescience in public health work which would appear hitherto to have been in large measure overlooked. As a matter of fact he was a pioneer in the investigation of the causes and possible prevention of infantile mortality, a subject which has bulked so prominently before the public of late years, and in reference to which, at the time of writing, further legislation is being sought from Parliament at the instance of the President of the Local Government Board.

Early in his official career, he was associated with the late Dr. Ballard in the investigation of mortality from diarrhœa among infants and young children; while in 1876 he was himself entrusted with inquiry into exceptionally fatal prevalence of this disease at Winchester. Here he found that, as in previous outbreaks, the majority of the cases came under observation in the third quarter of the year, while the incidence and mortality was almost entirely confined to children under five years of age. In view of the fact that, in other towns, excessive mortality from diarrhœa had been "found coincident with tainting of the atmosphere with the products of organic decomposition, especially of human excrement," detailed inquiry was made at Winchester as to the possible influences of similar conditions, while various social circumstances of the population (including the care and feeding of infants) were brought under review. As the result of his investigations Power was greatly impressed with the general helplessness and want of knowledge often exhibited in the upbringing of infants; and the need, with a view to safeguarding their elementary right to a chance of survival, for intelligent guidance and guardianship on the part of the State.

As the outcome of further study of the subject he realised the necessity for obtaining more accurate classification of the ages at which death occurred in the case of infants failing to survive for three months after birth, and with this object in view, shortly after being appointed Medical Officer to the Local Government Board, he took steps to procure periodical special returns of infant mortality from Medical Officers of Health throughout England and Wales.

Recognising also the need for detailed information as to the causes from which young infants perished, as well as their precise ages at death, it was mainly at his instigation that in the Registrar-General's Report for 1905 infantile deaths for the first time were classified as supervening at the following ages:—Under 1 week old, aged 1–2 weeks, 2–3 weeks, 3–4 weeks, 1–2 months, 2–3 months, and so on, up to 11–12 months.

Power had previously placed on record the disconcerting fact that, despite the application of science to the problems affecting public health, the amendment of the law with the object of furthering improved sanitary administration on the part of local authorities, and securing more adequate control of defaulting local authorities on the part of the Central Administration, the death-rate from "all causes" of children under one year old had, on the whole, remained practically unaltered for more than half a century, whereas the "all causes at all ages" rate had undergone steady reduction during the same period.

In this connection, moreover, he directed attention to the remarkable fact that if only the "all causes" infantile death-rate in England and Wales had improved *pari passu* with the death-rate for "all causes at all ages," this would have meant, during the quinquennial period 1898–1902 for instance, a saving of the lives of no less than 120,000 infants over and above those who actually survived; in other words, that there had been a loss of infant lives, from causes in all probability largely preventable, to the extent of no less than 24,000 per annum.

In this, as in other problems engaging his attention from time to time, he had no sooner elucidated to his satisfaction what appeared to constitute the main factors concerned in the annually recurring "slaughter of the innocents" than he set himself the further task of determining the lines along which progress might be expected to conduce to improved expectation of life in the case of infants, whether newly born or of later age. His recommendations to this end laid special stress on the necessity of the breast-feeding of infants or, where this was impracticable, or the child was already weaned, on the use of cows' milk the purity of which was ensured so far as possible by supervision of all the circumstances attending its production and distribution.

Among further recommendations he directed attention to the injurious effects likely to ensue on defective environment such as over-crowding and other conditions incidental to unsanitary housing accommodation. Remedial measures advocated by him, which have since received the endorsement of legislative enactments, include the notification of births, the training and appointment of health visitors, the co-ordination of voluntary organisations for Infant Welfare under the general supervision of the medical officer of health, and the control of milk supplies as well as, incidentally, other food supplies which are of essential importance to infants.

During his long connection with the Local Government Board he planned and directed a large part of the work of the Medical Department, including that comprised in the "Auxiliary Scientific Investigations" of which he was placed in charge when still a Medical Inspector, while of the numerous reports dealing with matters concerning the public health issued during his period of service many were either written by him or owed much to his editorial criticism and supervision. Nevertheless it is a somewhat curious fact that, owing to his retiring disposition, he was comparatively little known outside official circles. Indeed, his horror of publicity in any form or shape was so intense that he would neither attend meetings at which he might be called upon to speak, nor would he be photographed. The usual reference books knew him not, and even the 'Medical Directory' contained the barest modicum of information concerning him, until (almost certainly without his knowledge) the matter was taken in hand by one of his colleagues in the Medical Department.

As so felicitously expressed by Dr. McVail, on the personal side Power had a rare gift of friendship. His qualifications included a never-failing readiness to appreciate a point of view different from his own, and to throw his whole mind into the consideration and discussion of a question which was occupying the other man's thoughts. He used to say that, officially, his brain was a kind of gland through which all sorts of material had to pass for rejection or acceptance and digestion, and his half-amused grumble was that often it had not finished dealing with one substance before another was forcibly thrown into it. But this natural or acquired capacity for considering the most varied problems was always at the service of others, while the value of his help and criticism was enhanced by the remarkable faculty he possessed for mastering the principles and application of sciences which had been non-existent, or in a comparatively elementary stage, during his student days.

During his tenure of the post of Assistant Medical Officer, to which he was promoted in 1887, it became a well-recognised custom for any of his junior colleagues who might be at the office to drop into his room for half-an-hour or so after lunch, when, aided by the soothing influence of tobacco, opportunity was afforded for informal discussion of any points of interest or difficulty in connection with official work. Occasionally the conversation would take on a lighter or more personal tone.

The recollection of these gatherings and of Power's genial presence, quiet sense of humour, and keen interest in the doings of those who, in public health matters at any rate, were in a very real sense his pupils, will never be likely to fade from the memory of any of those now surviving who were privileged to participate in them.

In spite of frequent illnesses, due especially to attacks of influenza, he was a tireless worker and, as testified by an intimate friend, even insomnia was utilised as affording time to continue his labours. "The night cometh, when a man *can* work," was his explanation of how he accomplished so much, and his daily journeys from and to his house in Kent, or later on, in Surrey, were devoted to official papers. Only with the greatest difficulty could he be induced to take a holiday, owing mainly to an impression on his part that he was specially liable to attack by illness of some sort on his return to duty. Yet, as might have been gathered from his fine physique, he was something of an athlete in his youth, excelling in cricket and shooting, as well as in the navigation of a sailing yacht, his love for which pastime persisted to his later years.

Greatly devoted also to the study of Natural History, Power throughout an exceptionally busy life was wont to devote to this subject much of the scanty leisure at his disposal. And there can be little doubt that it was to his knowledge of Natural History that he owed the inspiration which resulted in his initiation of original lines of investigation, more particularly in regard to the possible and probable inter-relationship of disease of man and animals, to which reference has already been made. His special interest, however, lay in the systematic study, so far as available opportunities permitted, of the migratory habits of birds, concerning his observations of which he had kept records from 1858 onwards.

With his brother, Mr. F. D. Power, he gathered together the greater portion of a collection commenced by his father in 1840 and now housed by his cousin, Mr. Charles Cowper Mee, at Oldbury Hall, near Atherstone, Warwickshire, which includes no less than 250 groups of specimens of British birds. But he had no love for the mere annexation of specimens, and, equally, no sympathy with those who by indiscriminate shooting frightened birds away, and so disturbed the observations on which he was engaged. Among the specimens contributed to the collection by Power between the years 1862 and 1916, of which many were prepared by himself, certain specially rare examples, including a female hen harrier (*Falco cyaneus*) shot November, 1864; a merlin (*Falco æsalon*) shot in Kent, November, 1881; a specimen of a glaucus gull (*Larus glaucus*) captured at Stiffkey, Norfolk, November, 1887; and a garganey (*Anas querquedula*) shot, December, 1887, may be of interest to ornithologists.

Towards the end of his life, when physically incapacitated by illness, he still derived much pleasure from observing, with the aid of field glasses, and making notes as to the movements of birds that he could see from his garden at Molesey—swifts, swallows, martins, sand-martins, blackbirds, thrushes, willow-wrens, various tits and the black-headed gulls passing to and fro from Molesey Reservoir.

On the retirement of Sir George Buchanan in 1892, Power became First Assistant Medical Officer to the Local Government Board, succeeding to the post of Principal Medical Officer eight years later, on the death of Sir Richard Thorne.

In 1904, during his tenure of office as head of the Medical Service of the State, the Food Department was established on his initiative. He had long foreseen the value and importance to the country of the work possible of accomplishment as the result of such an expansion of the energies of the department under his control, and it will be generally admitted that his prevision has been amply justified, more particularly during the present war.

While Medical Officer to the Local Government Board, he also served, as Crown nominee, on the General Council of Medical Education, on the Royal Commission on Sewage Disposal, and on the Royal Commission on Tuberculosis, of which he subsequently became Chairman on the death of Sir Michael Foster.

He received the C.B. in 1902 and the K.C.B. in 1908, on retirement, at the official age limit. He was elected a Fellow of the Royal Society in 1895, and in 1907 was awarded the Buchanan Medal. Other honours awarded him include the Jenner Medal of the Epidemiological Society, the Bisset-Hawkins Medal of the Royal College of Physicians of London, the Stewart Prize of the British Medical Association, the Honorary Fellowship of the Royal College of Surgeons of England, and the Freedom of the Apothecaries Company.

Power will perhaps be best remembered for the somewhat unique faculty he possessed of communicating much of his own enthusiasm for research, and of his special genius for concentration on the essential details of whatever investigation he had in hand, to each of the fellow-workers with whom he successively came in contact. His, moreover, was a charming personality, which endeared him to all his colleagues, many of whom benefited to no small extent from his kindly help and encouragement, always so readily accorded. As regards his official work, it is not too much to say that no man in this country has done more than Sir William Power to advance the cause of scientific hygiene.

S. M. C.

CLEMENT REID, 1853–1916.

Clement Reid was born on January 6, 1853. His father was a goldsmith, and, as one of a large family, the subject of our memoir was compelled to depend upon his own exertions and at an early age entered a publisher's office, where he remained for six years. The work was distasteful to him, but in later life he acknowledged that he had benefited from the business training. His love for Nature was innate and as was appropriate he, a great-nephew of Michael Faraday, had that love intensified by the juvenile lectures at the Royal Institution. He determined to cut himself adrift from a business career and to devote himself to science, and, attracted to Geology, enthusiastically studied with the desire to obtain a post on H.M. Geological Survey. This he gained in 1874 and for nearly 40 years, to the day of his retirement in 1913, he was an officer of that Survey. From the time of his appointment until his death, he sedulously devoted himself to the pursuit of his science, with the success to which his published writings bear eloquent witness.

His work on the Survey began in the South-west of England, but he was soon transferred to the East Coast, and for many years laboured in Norfolk, North-East Yorkshire, Holderness, and Lincolnshire. He then moved south and mapped districts in the South Downs, Sussex Coast, Hampshire, the Isle of Wight, Dorset and Wiltshire. He was appointed District Geologist in 1901 and took charge of the Devon and Cornwall area, and afterwards, until the date of his retirement from the Survey, of the district around London. In 1908 he was sent to Cyprus on an Official Mission in order to advise the Colonial Office on the question of water supply.

Reid was elected a Fellow of the Geological Society in 1875, of the Linnean Society in 1888, and of the Royal Society in 1899. He was awarded the Murchison Fund of the first-named Society in 1886, and its Bigsby Medal

Photo by Elliott & Fry, Ltd.

Clement Reid

(1914)

in 1897; he also served for two terms on the Council of that Society and was a Vice-President in 1913–14. He also served for two terms on the Council of the Linnean Society. In 1911 he was awarded the Bolitho Gold Medal of the Royal Geological Society of Cornwall. He was a foundation member of the Société Belge de Géologie, de Paléontologie et d'Hydrologie.

He wrote much and on many subjects, but the work with which his name will be ever associated is that devoted to the Pliocene and Pleistocene deposits of this and other countries, with the consequent illuminating researches into the Pleistocene and later floras. For the conduct of this work he was naturally fitted by a happy combination of biological and geological knowledge, but, in addition to this, he displayed much fertility of invention and high manipulative skill, as evidenced by his published methods of extraction and preservation of plant remains from deposits of varying character.

A keen and accurate observer, he was likewise a sound reasoner, and was particularly averse to drawing inferences unless he felt that they were in the fullest degree justified by the facts. The foundation for much of his later work was laid while surveying on the Norfolk Coast, and the results are embodied in the classical memoir on 'The Geology of the Country around Cromer,' published in 1882. While studying the deposits of the Cromer Forest Bed Series he grasped the importance of the plants of this formation, and applied himself to the study of their seeds and leaves, thus preparing himself for those labours in Late Tertiary palæobotany, which he afterwards carried on, at first alone and then in collaboration with his wife, with eminent success.

When engaged in this work in subsequent years he contributed largely to our knowledge of the various Pliocene and Pleistocene floras of Britain, and, turning his attention to the Continent, gave us, with his wife's co-operation, the important series of memoirs dealing with the Pliocene flora of the Netherlands. In 1899 his knowledge was applied to the elucidation of the flora of these islands in the work entitled 'The Origin of the British Flora.' As one would naturally expect from Reid's two-fold training as naturalist and geologist, his work on the floras has been of great value both to the biologist and geologist. To the latter it has not only furnished evidence for a chronological sequence, but has also thrown much light on climatic changes in the past.

Reid paid much attention to the geological history of mankind, and, among numerous writings on this subject, special mention may be made of his important contribution on the relationship of Palæolithic Man to the Great Ice Age, in the Report on the Hoxne deposits, published in the 'British Association Report' for 1896.

As a stratigraphical geologist, his work ranged over many formations, but was mainly devoted to those of late geological date. The extent of his knowledge is shown by the 'General Memoir on the Pliocene Deposits of Britain,' which was written by him. When preparing this work he visited

Belgium and Italy in order to compare the deposits of those countries with their equivalents in England. Though essentially a stratigraphical geologist and palæontologist he contributed to all branches of geology, and, whatever might be his topic, he displayed his great originality.

He paid little attention to the popularisation of his subject, though eminently capable of the work, as witnessed by his delightful little book on 'Submerged Forests,' written for the Cambridge Series of Manuals of Literature and Science.

Reid was naturally of a retiring disposition, and may have appeared to some difficult of approach, and perhaps too determined in controversy. But those most intimately associated with him knew that this determination was due to his keenness for arriving at the truth. His opinions were ever formed from his own observations, where this was possible, and were not infrequently eminently original, and held with characteristic tenacity. It was not easy to be admitted into his circle of friends, but to those so admitted he gave of his best. They beheld one devoted to his science, giving his whole energy to it, and determined above all things to arrive at the truth. Nor was he so wrapped up in his science that he cared for naught else; an excursion in the field with him was not only an education but also a delight. His kindliness was constantly shown by the ever-ready help given to other workers.

When Reid retired from the Geological Survey in 1913, he made himself a home at Milford-on-Sea, in Hampshire, looking forward to a period in which he could follow his bent, free from official duties. He had already done much work in this retreat, and there, among other things, had begun, in co-operation with Mr. J. Groves, a research upon fossil Charæ, of which the first results were recently published. The period of retirement, however, was all too short, and he passed away quietly in the new home on December 10, 1916.

J. E. M. and E. T. N.

Photo by Maull & Fox

Daniel Oliver

DANIEL OLIVER, 1830–1916.*

By the death of Daniel Oliver Kew has lost its senior retired official, whose connection with the establishment began 1858. He was the first to bear the title of "Keeper of the Herbarium and Library," and was appointed in 1864.† He obtained the post through his own initiative. Under date of February 2, 1858, he wrote to Sir William J. Hooker, then Director of Kew, suggesting that possibly there might be an opening as Botanist to a Surveying Expedition, adding: "I venture to express a hope that thou wilt kindly afford me a chance of placing my best services at thy disposal." The result was an invitation to Kew, where he arrived in February, 1858. Deceased was born at Newcastle-on-Tyne on February 6, 1830, and was the son of Andrew and Jane Oliver, of Benwell Hills, members of the Society of Friends.

He was educated at the Friends' School at Brookfield, Wigton, where he early developed a keen interest in the study of Natural History in the field, and soon joined the Tyneside Naturalists' Field Club, now the Natural History Society of Northumberland and Durham. This brought him into contact with Sir Walter Trevelyan, Dr. Embleton and other enthusiastic North Country naturalists. Later Oliver became Lecturer on Botany in the Medical School of the University of Durham, and during these earlier years he made herborising excursions in the northern counties and in Ireland; always with a view to critical study and discovery.

His earliest publication‡ was, I believe, "On a Few Plants found in Bouldersdale and Teesdale, together with the Formations on which they were Found," which appeared in the 'Phytologist,' vol. 2, 1847. This was followed, previous to his going to Kew, by a number of short papers, mostly on British plants. Notable in his early herborisings was the discovery of *Naias flexilis* in Connemara, thus adding a new genus to the Irish and British flora: a fresh water organism of wide distribution, which has since been recorded within the United Kingdom from Scotland. Meanwhile, in 1851, he had been elected a Member of the Edinburgh Botanical Society; in 1853 he entered the Linnean Society of London, of which Society he was the Father at the time of his death, with six years' seniority over Sir Howard Elphinstone and Sir John Llewelyn, the former of whom followed Oliver less than a fortnight later.

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* This sketch is in part a repetition of my memoir, with portrait, which appeared in the 'Journal of the Kew Guild' for 1898; in part from information kindly supplied by the deceased's family; in part from particulars extracted from the 'Kew Bulletin, supplemented by my personal knowledge of Prof. Oliver's career.

† His lamented predecessor, A. A. Black, became "Curator" in 1853, and died in India in 1864; but his was not a Civil Service appointment.

‡ A bibliography of Oliver's botanical work is given in the 'Kew Bulletin,' 1917, pp. 32–36.

As already stated, Oliver entered Kew in 1858, when funds were low and Sir William Hooker was struggling in the face of great difficulties to lay the foundation of a scientific botanic establishment, in this he was ably assisted by his son, J. D. Hooker, and Daniel Oliver. At this period Hooker's private herbarium and portions of his unrivalled private library were housed in the old part of the present range of Herbarium buildings, together with the collections and books presented to the nation by George Bentham, William Arnold Bromfield, and others. Dr. J. D. Hooker's Australasian, Antarctic, and Indian collections, as well as those of William Griffith and several other notable travellers, were in process of classification and elaboration. This involved an immense amount of mechanical labour which Oliver took up with untiring energy in return for a mere pittance. In 1859 he inaugurated a free course of lectures on Botany to the young gardeners of Kew Gardens, and this was supplemented in 1860 by instructional evenings devoted to elementary chemistry, electricity, meteorology and meteorological instruments, varied by readings of selections from the Kew correspondence of Gustav Mann, Dr. F. Welwitsch, and others. He continued the lectures on Botany until 1874. The writer's acquaintance and official association with Prof. D. Oliver began in the autumn of 1860, and most of what follows is given from personal knowledge.

Fortunately for Oliver, his meagre Kew stipend was soon augmented in a substantial manner by his appointment in 1861 in succession to Dr. Lindley to the Botanical Chair of University College, London, which he occupied until 1888. During a number of terms I had the advantage of acting as his preparer, for which I was liberally remunerated, besides adding to my very slender stock of general knowledge. In this connection let it be mentioned that every hour, indeed every quarter of an hour, borrowed from his official time at Kew was repaid to a minute. But he was scrupulously conscientious in all things, and a disciplined example to his subordinates in punctuality and other qualities that make an effective and respected leader.

Returning to his published work, after his settlement at Kew; only a small selection can be noticed. He was the principal contributor on Botany and Assistant Editor of Busk's 'Natural History Review,' 1861–65. It was in this serial that his paper appeared on "The Atlantis Hypothesis in its Botanical Aspect," a paper that created unusual interest at the time. The paper was written in controversion of Heer and Unger's hypothesis that during the Miocene Period there existed an Atlantic junction between Europe and America. Oliver's arguments against this hypothesis were based on known facts of the recent distribution of plants and a critical traverse of Heer and Unger's identifications. Asa Gray's comparison of the floras of eastern North America and Japan is also quoted by Oliver in support of his position. No serious defence of Heer and Unger has been attempted, I believe; and Unger's 'New Holland in Europa,' of about the same period, was adversely criticised by George Bentham and indirectly by Oliver.

Many of Oliver's papers of great interest on Systematic and Geographical

Botany appeared in the publications of the Linnean Society. Prominent among these are annotated lists of plants collected in various remote and then little known parts of the world, including the mountains of eastern tropical Africa, certain Pacific islands, and the Arctic regions. Of the papers of greater length, the "Botany of Speke and Grant's Nile Expedition," the "Botany of Everard im Thurn's Roraima Expedition," and "List of the Plants Collected by H. B. Guppy in the Islands of Bougainville Straits" are noteworthy examples. Many of these collections were from fresh territories or little known regions and contained numerous highly interesting new generic types, especially West Tropical African and Malayan collections. But, like the herbarium, Oliver's work embraced the phanerogamic flora of the world in all its families, and the more complex or difficult families such as the Olacaceæ, Hamamelidaceæ, Flacourtiaceæ, Utriculariaceæ, and Loranthaceæ, had a special attraction for him. Begonia was a favourite genus, and he had the good fortune to discriminate Begoniella of the Andes and Hillebrandia, peculiar to Hawaii, the only other genera commonly admitted in the Begoniaceæ.

Oliver was author of the first and several succeeding editions of the Official Guide to the Kew Museums and also of the 22nd to the 30th Edition of the Official Guide to the Royal Gardens and Pleasure Grounds, Kew, 1863–85. These, as well as his educational books, are written in simple yet clear language, within the grasp of the multitude. His lectures were equally lucid, though not of an oratorical character. He adopted the deceased Prof. Henslow's type method of teaching Systematic Botany by the use of schedules, and his little book, 'Lessons in Elementary Botany,' embodies the principles of this method, preceded by chapters on Elementary Structural and Physiological Botany. There are many editions of this admirable primer; the first appeared in 1864 and the last in 1910. His 'First Book in Indian Botany' is on similar lines and has run through nine impressions, 1869–1911. His 'Illustrations of the Principal Natural Orders of the Vegetable Kingdom' deserves special mention in this connection. It contains upwards of 100 excellent hand-coloured plates by W. H. Fitch, with dissections and explanatory letterpress. The first volume of Oliver's 'Flora of Tropical Africa' was published in 1868, and the work was advanced by him, assisted by other Botanists, to the third volume, in 1877, when stress of other official work and lack of assistants prevented its continuation.

For a number of years Oliver edited and was almost sole contributor to Hooker's 'Icones Plantarum,' in which he published many novelties detected in various collections from nearly all parts of the world, including A. Henry's earlier discoveries in Central and Western China. I may here relate an incident in this connection. Among A. Henry's novelties were specimens bearing leaves like those of an Æsculus and terminal clusters of flowers of the ordinary Viburnum structure. This singular combination was described and figured by Oliver as a new genus under the name of Actinotinus. Some months later Oliver came upon another puzzle in which papilionaceous flowers were associated with a type of foliage quite new to the Leguminosæ. He showed the specimens to Mr. N. E. Brown and the writer, and the critical-eyed Brown at once exclaimed " Why, the flowers are inserted in a branch of something different." It flashed across Oliver's brain that John Chinaman had deceived him and he hastened to fetch the specimens of his Actinotinus, when the fraud was evident; but it was so cleverly manipulated that it had escaped detection by at least four persons who had handled the specimens. I never before or since saw Prof. Oliver so excited, and I am sure it disturbed his truth-loving soul for days, if not weeks. I am not sure whether he ever knew that I was similarly victimised some years later, when I described the leaves of a Daphniphyllum and the flowers of a Rhododendron as a new species of the latter genus. But I do know that the same Chinaman tried the same trick with Mr. E. H. Wilson; was detected and mulcted of three weeks' wages.

Allusion has already been made to Oliver's artistic temperament, but nothing has been said of his activity in Art and its development, and how he became acquainted with Ruskin. In 1870 he delivered a course of 10 lectures on Botany at South Kensington Museum, in which the doctrine of "axis and appendages" was expounded. Meeting a lady at tea one day, Ruskin asked her what she had been doing, and she explained that she had attended these lectures, and informed him that the lecturer had stated that there were " seven sorts of leaves, and that there were no flowers." Ruskin used this as his text for a diatribe in 'Fors Clavigera.' Apparently, Oliver expostulated, for later in the same volume there is an apology. However, the sequel was a friendly visit to Kew and a lifelong friendship as the result.

Oliver's holidays were largely spent in sketching, and his 'Plant and Animal Forms as used by Workmen of the Middle Ages in Decoration, chiefly of French Churches,' was the outcome of a sample of his work of the period 1882–86. The selection consists of 50 large quarto sketches, privately reproduced in lithography, and distributed among his friends. This work was highly praised by Ruskin, though he unfavourably criticised the drawings in the fifth " Decade." The writer possesses a set of this work, given him by the artist, which comprises examples of the stonemason's art at Senlis, Noyon, Amiens, Laon, Coucy, Soissons, Beauvais, Provins, Troyes, and Chartres. It has a special interest now, as several of these places are within the area of destruction. After his retirement from the Service in 1890, Oliver successfully devoted his leisure to gardening and painting in oil.

Our botanist had hobbies, and he began collecting examples of the illustrated works of the "old masters" in botanical literature at a period when *editiones principes* were to be had at moderate prices, and his collection comprised choice copies of the most celebrated authors. In this matter he may have been influenced by William Arnold Bromfield's legacy to Kew, which included a small but critical selection of herbals.

Oliver's activity was by no means confined to that which bears his name.

He was consulted by Bentham and the Hookers on all knotty points which arose in connection with the compilation of the great 'Genera Plantarum' and other Kew publications. No botanist possessed a more profound knowledge of the classification and geographical distribution of the phanerogamic elements in the vegetation of all regions of the earth; none was better versed than he in the structure of the "formæ abnormes" and "genera anomala," yet he was too conservative and too modest, or perhaps too faithful to the Kew traditions, to give the world his own ideas of an amended classification. Perhaps this was due to the fact that his soul was more enchanted by the beautiful forms of nature than by their arbitrary groupments for purposes of study. His love of the beautiful was exemplified by his matutinal button-hole of flowers, regularly transferred to a little vase on his work table. As a worker he was very rapid; all his actions were nervously performed, yet controlled by an equally spontaneous thinking power. Still, it must be admitted that his scattered descriptions of interesting novelties are sometimes neither so full nor so instructive as he could have made them.

By nature, Oliver was hostile to personal honours in the form of medals and other emblems for distinguished services which he regarded as duties to God and man. Yet he had perforce to receive a Fellowship of the Royal Society in 1863, a "Royal" Medal (1884), and the Linnean Gold Medal (1893). When he retired from the public service in 1890, H.M. First Commissioner of Works placed on record the high appreciation of the Government of the valuable services rendered by Oliver to the Royal Botanic Gardens, Kew, and the distinguished ability which he had brought to bear on the work of his department. In 1891 the University of Aberdeen conferred upon him the honorary degree of LL.D. A portrait of Oliver by J. Wilson Forster was presented to the Kew Herbarium by his friends and admirers in 1893.

In July, 1916, there were four successive keepers of the Kew Herbarium and Library living, and they were photographed in a group in Mr. J. G. Baker's garden at Kew. They were: Daniel Oliver, in his 87th year; John Gilbert Baker, 84th year; William Botting Hemsley, 73rd year; and Otto Stapf, 59th year. The association in a picture of four successive living holders of the same office is such an unusual event that it seems of sufficient interest to be put on record here.

The foregoing lines inadequately commemorate the life of an esteemed friend, a generous colleague, a faithful public servant, and an accomplished man; but they are the words of a grateful pupil and admirer.

The portrait by Maull and Fox was taken in 1903.

W. B. H.

CAPTAIN GEOFFREY WATKINS SMITH.

BY the death in action on July 10, 1916, of Captain Geoffrey W. Smith we have lost one of the most brilliant of the younger generation of zoologists. He was born in 1881, the youngest son of the well-known Westminster magistrate, Mr. Horace Smith, and Mrs. Smith, of Ivy Bank, Beckenham. Starting his school career at Temple Grove School, he went to Winchester College, and afterwards to New College, Oxford, where he was given a scholarship in 1900. After taking his degree in Zoology, he devoted himself to research and to teaching both in his College and in the Department of Comparative Anatomy in the University Museum. In 1907 he was elected Fellow and Lecturer of New College, and some years later became Tutor. When the war broke out he volunteered his services, joined the O.T.C., and soon obtained a commission in the Rifle Brigade. He was killed by a shell in a trench just captured from the Germans, near Pozières, in France.

Geoffrey Watkins Smith will be deeply regretted by all who knew him. His was a particularly charming personality, a rare combination of simplicity and youthful gaiety, with a refined and truly cultured mind; "one of those bright and joyous spirits whose presence seems to shed happiness." Of a most lovable character, of unfailing good humour and courtesy, he endeared himself to all those with whom he came in contact. His pupils and colleagues in Oxford were no less devoted to him than the men he led at the Front. Geoffrey Smith was a man of wide interests and keen appreciations, yet his learning sat lightly on him. He was no recluse, and thoroughly enjoyed the good things of life. Fond of all kinds of sport, he excelled especially in golf and lawn-tennis, where his elegant figure and graceful movements showed to the best advantage. He delighted in good literature, whether English or foreign, prose or poetry, and himself was the author of a little volume of charming Christmas carols and of other poems, having, doubtless, inherited his literary gifts through his father. Indeed, the excellent style of his scientific writings adds much to their value.

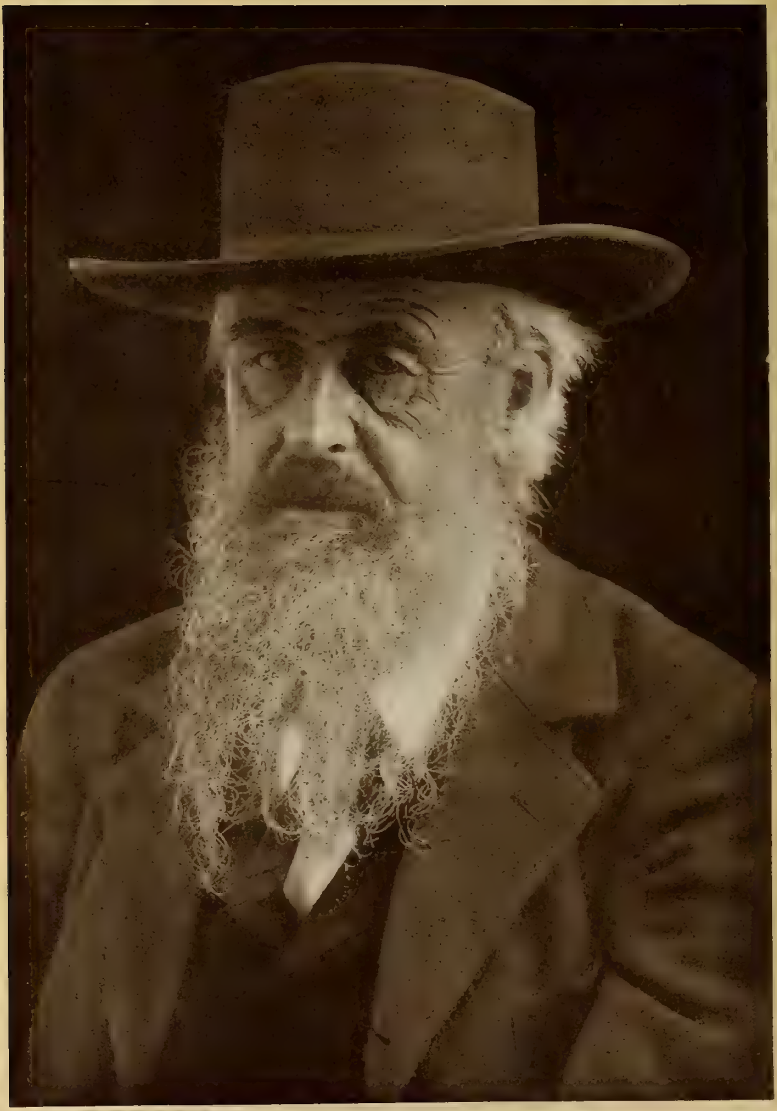
Geoffrey Smith early became interested in natural history, and as a mere schoolboy contributed notes on birds to the 'Zoologist' (1898). Before taking his degree he paid a short visit to Prof. R. Hertwig's laboratory in Munich, where he completed some work on "The Mass Relations of Nucleus and Cytoplasm in Actinosphærium" ('Biometrika,' vol. 2, 1903). Although his next paper was an embryological study "On the Development of the Columella Auris in Birds" ('Quart. Journ. Micr. Sci.,' 1904), he soon became absorbed in the study of the wider problems of heredity and evolution he met in his investigations on sex and parasitism. Having completed his course at Oxford and obtained the University Scholarship at Naples, he went out to Italy in 1903, and devoted his attention more especially to the

Crustacea. In a short paper "On the Metamorphosis of Gnathia" ('Mitth. Zool. St. Neapel,' 1904), he corrected and completed our knowledge of the life-history of this strange parasite, and shortly after he described among male Tanaidæ and other Crustacea a form of high and low dimorphism comparable to that already known among insects. At the request of Prof. Dohrn he then undertook to write a monograph on the Rhizocephala, a group of parasitic Cirripedes of extreme interest. For this purpose he remained for nearly three years in Naples, and produced a monograph which, although one of the shortest, is certainly one of the most interesting of the whole series. Indeed, it is typical of the scientific work of Geoffrey Smith, who aimed neither at encyclopædic completeness nor at the accumulation of meaningless detail. Bringing to bear on the problems before him a mind of remarkable freshness and originality, he attacked them from new points of view and with the help of new methods, never losing sight of the main points at issue.

Since this monograph, together with a series of "Studies in the Experimental Analysis of Sex," published later in the 'Quart. Journ. Micro. Science,' 1910–14, formed his most important contribution to science, they deserve special notice. The life-history of the interesting Rhizocephalan Sacculina, a parasite which infects crabs, had already been worked out, chiefly by Delage, and Geoffrey Smith was able not only to confirm the brilliant work of the French zoologist, but also to complete his observations, especially with regard to stages between the larva which has just penetrated into the host at any point, and the young Sacculina fixed in its definitive position on the intestine of the crab. Some time previously Giard had described the remarkable effect produced on the host by the Sacculina, and named it parasitic castration. It results in the profound modification of the sexual characters of the crab, the degeneration of its testis or ovary, and the acquisition by the male of the secondary sexual characters of the female. The difficult problem of the feminisation of the male Smith attacked with conspicuous success. He showed that the female, if parasitised young, was merely made to acquire prematurely the characters of the mature adult; that the male only has the capacity to acquire the characters of the opposite sex, that a male individual, having got rid of the Sacculina, and recovered from its attack, regenerates an hermaphrodite gonad with ova and spermatozoa. This modification of the sexual characters, he argued, is related to a deep-seated change in the general metabolism. In the maturing female fat metabolism is very active for the purpose of nourishing the eggs, the blood becomes loaded with fat and the liver stored with it. The parasite, absorbing food-material, plays the same part in the host as the ripening ovary, stimulates fat production, and thus converts the metabolism of the male to the female type of metabolism. Since the secondary sexual characters appear before the regeneration of the gonad, it follows that in this and in many other cases the development or modification of the characters is due, not to the secretion by the ovary or testis of some special hormone, but to a profound alteration in the metabolism of the animal.

In fact, from these researches and experiments made on birds and frogs, Geoffrey Smith became convinced that the existence of a "reproductive hormone" was unproved. Very ingeniously he reconciled the fact that among the Crustacea the male only shows signs of hermaphroditism with a Mendelian scheme of heredity by suggesting that the female is a homozygote, and the male a heterozygote in respect of sex factors. Smith seems to have been the first to formulate such a theory, and it is interesting to note that it is along these lines that the most successful work on the Mendelian interpretation of sex has since developed. He conceived that it is by the co-operation of these factors of heredity with certain products of metabolism that sexual characters develop, the presence of both being necessary. Other interesting conclusions followed from his work on the Rhizocephala. For instance, he was led to the view that sessile and parasitic Crustacea are often hermaphrodite because of the change in their metabolism due to their peculiar mode of life, that such hermaphrodites have all been derived from the male sex, the female having been suppressed, and, further, that the so-called complementary males of Cirripedes, described by Darwin, are in reality arrested protandric hermaphrodites of the same nature as the large individuals on which they become fixed.

In 1907 he undertook a journey to Tasmania, chiefly with the object of studying Anaspides, a strange fresh-water Crustacean recently discovered there. On this expedition, of which he gave a delightful account in a little book entitled "A Naturalist in Tasmania," he not only obtained Anaspides, but also a related but quite new genus Paranaspides. With the help of this material he was able to bring forward convincing evidence of the correctness of Dr. Calman's contention that the Anaspidacea are the remnant of a once widely distributed group found in Carboniferous strata, and worthy of being placed in a separate division, the Syncarida. He also collected other forms on his travels, and published monographs on the land and fresh water crayfishes of Australia, in which the geographical distribution, habits, and inter-relationships of the species are discussed with his usual skill and originality ('Proc. Zool. Soc.,' 1912 and 1913). Further, we owe to Smith the excellent account of the Crustacea in the 'Cambridge Natural History' (1909). Quite recently have appeared a paper on "The Genus Lernœodiscus" ('Journ. Linn. Soc.,' 1915) and a continuation of some work begun with G. H. Grosvenor, on the reproduction of water fleas ('Proc. Roy. Soc.,' 1915). The appearance of sexual forms in the life-cycle of these Cladocera reproducing parthenogenetically is here shown to be not necessarily rhythmical and entirely due to internal causes as Weismann supposed, but capable of being suppressed or encouraged by external conditions, and subject to experimental control. Evidence is also brought forward that rapid growth and parthenogenesis are related to glycogen storage, while sexual reproduction is related to fat storage, differences in metabolism being at the root of the antagonism between growth and sex. Other problems are dealt with in the "Studies," such as the parasitism of bees by the insect Stylops, and the



H Graf z Solms Laubach

sterility of hybrid birds due to abnormal spermatogenesis and the development of giant spermatozoa.

Smith seems to have possessed "the gift of penetrating the secrets of Nature." As in play so in work, he never wasted time or strength. Preferring the simplest methods, he worked rapidly and reached his conclusions quickly. Yet when needful no one was more careful to scrutinise results and test them with the use of the best technique and most scrupulous impartiality.

Several of his works were written in collaboration with others, for he was an excellent teacher, and always most successful in inspiring his friends and pupils with some of the enthusiasm he felt so keenly himself.

E. S. G.

HERMANN GRAF ZU SOLMS-LAUBACH, 1842–1915.

HERMANN GRAF ZU SOLMS-LAUBACH was born on December 23, 1842, at Laubach, in Upper Hesse, the seat of his branch of the ancient family of Solms, which traces its descent to Marquard, Graf zu Solms im Oberen Lahngau, in 1129. His parents were Graf Otto zu Solms and the Gräfin Luitgard, Princess of Wied.*

On leaving school, Solms wished to pursue a learned career, but his father objected, not, as has sometimes been said, on family grounds, but because he did not consider his son sufficiently gifted. It appears that he had not done uniformly well at school. However, he was eventually allowed to enter at the University of Giessen; at that time he was still in doubt whether to take up History or Natural Science. It was Leuckart's lectures on Comparative Anatomy which attracted him to the latter. That he ultimately followed Botany rather than Zoology was due to his early surroundings, for both his uncle and his eldest brother were botanists, and Solms' interest in the subject began when he was still a boy.

After one term he left Giessen for Berlin, where he attended Alexander Braun's lectures on Botany. He afterwards spent a term with de Bary at Freiburg (the beginning of an association which influenced his whole life), but it was at Berlin that he took his Degree in 1865. His dissertation was on a parasitic plant, the Toothwort (*de Lathræœ generis positione systematica*), a line of work which occupied him much in later years.

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* For many details of Solms' life the writer is indebted to the obituary notice by his successor, Prof. Jost, in 'Berichte der Deutschen Botanischen Gesellschaft,' vol. 33, pp. 95–112 (1916).

After a visit to Geneva, to study the famous collections there, he made a scientific journey to South Portugal, where he specially interested himself in the distribution of mosses. He then returned to work with de Bary, following him on his removal to Halle, where Solms became *Privatdocent* in 1868.

As regards Solms' part in the war of 1870, Dr. Lotsy, an old pupil of his, writes: "As far as I am aware . . ., he served as a Johanniter, viz., as one whose sole duty is taking care of the wounded."

In 1872 Solms became *Professor Extraordinarius* at the new German University of Strasburg. He remained there, as a colleague of de Bary's, till 1879, when he was appointed full Professor at Göttingen, succeeding Griesebach. In 1883 he visited the Tropics, working, at Treub's invitation, in the new laboratory at Buitenzorg.

At the close of 1887 Solms was invited to occupy the Chair of Botany at Berlin, as successor to Eichler. After much hesitation he accepted the call, but the death of de Bary in January, 1888, caused him to reconsider his decision ; he preferred in the end to succeed his old friend and teacher, and became Professor and Director of the Botanic Garden at Strasburg. He continued to hold this post for 20 years, and, after his retirement, remained at Strasburg till his death. He passed away peacefully on November 24, 1915, his mental powers, in spite of much bodily weakness, retaining their activity almost to the last.

Solms-Laubach's original work as a botanist covers a remarkably wide area. He began as a collector of plants in the field, and retained his love for botanical excursions all through life. Prof. Jost quotes an amusing passage from a letter from Alexander Braun to de Bary, introducing Solms: "Wenn Du ihm das verdammte Exkursionenmachen abgewöhnen kannst, wird vielleicht etwas ganz brauchbares aus ihm." As a matter of fact, the excursion habit made him a sound systematist, gave him his keen interest in the geography of plants, and laid the foundation of his greatness as an investigator.

One side of his work throughout life was purely systematic; he monographed a number of families for de Candolle, Engler, and other editors. Among these families several (Rafflesiaceæ, Hydnoraceæ, Lennoaceæ) are parasitic Phanerogams, a biological group of extraordinary interest, on which Solms was probably the leading authority. His earliest (1863), and almost his latest work (1914), was on parasitic plants, and to his elaborate anatomical and morphological researches a great part of the existing knowledge of these strangely modified forms is due.

Other systematic publications stood in close relation with Solms' work on the history of cultivated plants, another favourite subject of his. The Figs, the Papaw, Wheat, Tulips, and Strawberries were all investigated by him ; the connection of cultivation with History and Philology, studies in which he never lost his interest, was a special attraction to him. His attention was not much directed to the Genetics of cultivated plants, which would now seem the most promising line of research, although we are informed by Jost that Solms knew Mendel's work well, long before its rediscovery in 1900.

In other connections, however, Solms showed a keen interest in the question of the origin of species. His studies on Cruciferæ, for example, started with the description of *Capsella Helgeri* (1900), a form with indehiscent fruits, which, on the ground of cultural experiments, he regarded as a mutant of the common *C. Bursa-pastoris.*

His interesting and original treatise on the geography of plants (1905) opens with a discussion of the origin of new forms, in which he shows himself much influenced by the views of Nägeli, a writer whose theories on the subject have never much appealed to evolutionists in this country. The latter part of the book, dealing directly with distribution, and based largely on his own observations in the field, is of much higher value.

In addition to the work on parasites already mentioned, Solms carried out a number of morphological researches extending to all classes of plants. Among Fungi, he published on *Empusa* (1869), *Penicilliopsis* and *Ustilago* (1886), but his works on Algæ are of far greater importance. Quite early, in 1867, he elucidated the reproduction of *Batrachospermum*, and founded the genus *Janczewskia*, for some very remarkable epiphytic Florideans. In his monograph of the Corallineæ of the Gulf of Naples (1881), he cleared up the complicated reproductive processes in this important family. At a later date he extended his researches to calcareous Algæ of widely different affinities (Siphoneæ), publishing in 1892 a paper on the genera *Cymopolia, Neomeris*, and *Bornetella*, while in 1895 the Linnean Society of London had the honour of bringing out his monograph of the Acetabularieæ, a most important work, dealing with fossil as well as recent forms, and the only memoir of his published in this country, and in English.

Among the Bryophyta, he wrote on some of the less known families of Marchantiaceæ (1897 and 1899), and, of recent vascular Cryptogams, he investigated *Psilotum* (1884), especially its propagation by gemmæ, and, in later years, the branching of *Isoëtes* (1902). His paper on the morphological construction of *Stangeria* and other Cycads (1890) is well known, and of equal importance for recent and fossil botany. Of his morphological researches on Angiosperms, that on monocotyledonous embryos with an apical growing point (1878) is of quite special interest.

Physiology he scarcely touched on, unless we put under this head observations on the occurrence of calcium oxalate in the cell-wall (1871).

We now come to Solms' work in Fossil Botany, the field in which he undoubtedly exercised the strongest influence. Some interesting remarks by Prof. Nathorst on Solms' position as a palæobotanist are quoted in Prof. Jost's obituary notice. Prof. Nathorst points out that the leading idea in all Solms' work on the subject is that fossil, like recent plants, have to be considered from a purely botanical point of view. Previous writers, to use Solms' own expression, "had to serve two masters, Palæontology and Botany." In Solms' handbook the botanical results are treated independently, and for the first time made accessible to botanists in a connected form, The significance of palæobotany from a developmental and systematic point of view thus became self-evident. Solms conceived the object of his work to be the completion, from fossil data, of the Natural System of Plants.

The 'Einleitung in die Palæophytologie,' published in 1887, and translated into English four years later, under the title 'Introduction to Fossil Botany,' certainly made a great impression on botanists, and to many was the beginning of their interest in the fossil record. The present writer was among these, and well remembers how the reading of Solms' book, on its first publication, revealed to him, what he had never quite realised before, that fossil plants really matter to a botanist. It prepared him to take an intelligent interest, at a later date, in the things themselves. The book owed its influence entirely to its solid merits as a botanical exposition of the fossil evidence. It is inadequately illustrated (though the figures are well chosen) and Solms' style was seldom attractive.

The 'Einleitung' appeared early in Solms' career as a palæobotanist, at a time when he had published little original work on the subject.* Hence it may seem to the present-day reader to err on the side of over-caution. For example, the Calamarieæ, though he strongly inclined to Williamson's view of their affinities, are widely separated from the Equisetaceæ, the whole of the Lycopod series intervening.

The important palæobotanical researches which followed up the publication of this book were in many cases the outcome of preliminary work done in the course of its preparation. The book may be said to have built its own tomb, for owing to the mass of new work to which it gave the stimulus, it soon became out of date, and Solms could never be induced to consider a second edition. Its historical interest as a classic will always remain.

Solms' special work in Fossil Botany must be dealt with very briefly, though it merits a full review. Much the greater part is on remains showing structure; in the early and admirable memoir on Permian Coniferæ (1884), great stress is already laid on anatomical data.

Solms' book contains an excellent account of *Bennettites,* in which he had already recognised the embryo. This was followed up, in 1890, by the publication of his well-known paper on the fructification of *B. Gibsonianus*, completing the work of Carruthers, and clearing up doubtful points. This investigation was on English material, and the paper was accordingly translated in the 'Annals of Botany' for the following year. An important memoir, written in Italian, in collaboration with Capellini, on the Italian specimens of Bennettiteæ, was published in 1892. It was here that Solms first found evidence of the bisexual character of the Bennettitean fructification, foreshadowing the brilliant discoveries of Wieland.

In the same year Solms began a series of papers on the Lower Carboniferous plants of Falkenberg in Silesia (1892–1910). The first part deals chiefly with *Zygopteris* and *Lepidodendron,* in which he found the ligule, discovered just before in another species, by Hovelacque. Part II (1893) is devoted to the isolated type *Protopitys* (Cycadofilices), of which he first elucidated the structure. The third part, 1897, gives a full account of the anatomy of *Archæocalamites*; while the fourth (1910), appearing after a long interval, is perhaps the most important of all, describing those remarkable polystelic Cycadofilices, *Völkelia, Cladoxylon Kidstoni,* and *Steloxylon.*

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* In the introduction to his paper on Permian Coniferæ (1884) Solms speaks of himself as "Homo novus" in the field of Palæophytology.

In the meantime Solms published another paper, of great importance, on plants of a similar horizon, namely, Unger's specimens from Saalfeld (1896). Our knowledge of the genera *Cladoxylon* and *Calamopitys,* besides many other forms, is to a great extent based on this work, to which the present writer is especially indebted.

In 1894 he described the structure of *Stigmariopsis,* perhaps representing, as he suggested, the underground organs of the smooth-barked Sigillarias.

The memoir on Middle Devonian plant remains from the Lower Rhine (1895) is of interest from the age of the fossils. The specimens were, however, fragmentary. It is worth noting that Solms at that time recognised Dawson's *Psilophyton princeps* as a distinct and important type, though sceptical as to its supposed fructification. It is only within the last year that the work of Halle and of Kidston and Lang has established the group Psilophytales on a firm basis and justified Dawson's conclusions.

In the same year Solms gave an admirable account of a new type of *Sphenophyllum* cone, under the name *Bowmanites Römeri,* and added much to our knowledge of the family.

The work on *Medullosa Leuckarti* (1897) is a valuable contribution to the anatomy of one of the most remarkable groups of Palæozoic plants. The existence of a class of plants, including *Medullosa,* intermediate between Ferns and Cycads, suggested by Williamson, was first recognised by Solms, as far back as 1887.

In 1899, besides a work with Steinmann on the Rhætic Flora of Chili, Solms published an account of the Triassic genus *Pleuromeia,* probably a late representative of *Sigillaria.*

His work on the petrified plants of Franz Josef's Land, in the Arctic regions (1904), though the fossils were botanically of no great interest, enabled him to determine the age of the Flora as approximately that of the Wealden.

Of his later fossil works, the chief is a paper with the singular title "The Deep Black *Psaronius Haidingeri,* of Manebach, in Thuringia" (1911). In this he explained for the first time the true nature of the zone enclosing the adventitious roots of *Psaronius,* and showed that it is not part of the cortex, as was generally supposed, but a dense felt of hairs, springing partly from the stem, partly from the roots themselves. He thus confirmed an opinion expressed some years before by Farmer and T. G. Hill.

Solms' last fossil paper was on "*Tietea singularis,* a New Fossil

Pteridineous Stem from Brazil" (1913). He compared the specimen with *Psaronius*, but there seems to be some doubt whether he rightly interpreted its structure.

Two lectures by Solms may be mentioned here, both given before a Strasburg Society. The first (1906) was on the Significance of Palæophytology for Systematic Botany. He points out, with admirable clearness, the bearing of fossil evidence on evolution, especially as confirming the great morphological discoveries of Hofmeister. He dwells in particular on the connection between Ferns and Cycads, and on the geological history of the main groups of Ferns. The other lecture was given at a celebration in 1909 of the 100th anniversary of Darwin's birth. While Solms speaks with warmth and just appreciation of Darwin himself, he summarily dismisses the theory of Natural Selection, saying, "To-day it is doubtless wholly given up by almost all botanists and by many zoologists." Thus Solms, like Zeiller, was an Evolutionist, but by no means a Darwinian. In his depreciatory estimate of Darwin's great theory, the influence of Nägeli can be clearly traced. But it is true that the Darwinian period was beginning to wane by the time that the celebrations were held.

All Solms' original work was sound and accurate in the highest degree, and it covered probably a wider field than that of any contemporary botanist. His memoirs, however, are by no means easy reading; the style is dry, and it is sometimes difficult to extract the important results from the mass of detail. He was personally an infinitely more interesting man than one would imagine from reading his special works.

His real character comes out better in his reviews, which are often trenchant and piquant. It is quite worth while to look through them in the pages of the 'Botanische Zeitung' and 'Zeitschrift für Botanik,' which he edited for so many years.

As a teacher Solms must have been impressive and stimulating. The writer once had the good fortune to hear one of his elementary lectures at Strasburg; the subject was Phyllotaxis and Branching. His manner of lecturing was striking and somewhat eccentric; all the time he was pacing up and down like a caged lion, and making free use of his arms, in a way that all his friends will recall. The lecture was clear and vigorous; he took great pains with the elementary course.

Dr. Lotsy writes: "I think I can state with justice that I never have had a better teacher than him, in the years I spent at Göttingen. Solms had very curious peculiarities in lecturing; it even happened that in his enthusiasm the lamps above his catheder came down, but he knew how to rivet our attention to such a degree that such things were hardly noticed by us."

In the laboratory Solms' great principle was to let his pupils work out everything for themselves. He complained of English students for not being independent enough in their work.

In private life Solms was a delightful companion, always interesting and amusing, and with a wealth of knowledge of all kinds rarely equalled. He was a great traveller and endowed with a wonderful memory, so that he was full of intimate reminiscences of many lands. Jost tells us how a Russian friend said of him, "He knows Moscow better than I do." The writer can say the same of Solms' knowledge of this country. He was often in England, where his tall figure and striking features were familiar at many scientific meetings; on his first visit, in the sixties, he was a guest of Sir William Hooker's at Kew. His English was fluent, but original. Some of his best friends were Englishmen; in particular he was much attached to the late Prof. W. C. Williamson, whose work he was the first to appreciate. To those whom he found congenial—and he was not hard to please—Solms was an absolutely true and faithful friend, generous and open-hearted.

When in England he took a great interest in the country and its Natural History, as well as in the people. He was much impressed by the wild box trees on Box Hill, and when in Hampshire a few years ago showed great pleasure at seeing bluebells (*Scilla nutans*) wild for the first time in his life.

Solms received various marks of recognition in this country; he was elected a Foreign Member of the Royal Society in 1902, and received a similar honour from the Linnean Society in 1887, the Royal Microscopical Society in 1895, and the Geological Society in 1906. The Honorary Degree of Sc.D. was conferred upon him by the University of Cambridge at the Darwin Celebration in 1909. He was awarded the Gold Medal of the Linnean Society in 1911, coming over in person to receive it, an occasion on which he made a graceful speech in returning thanks.

Solms was so well known and appreciated in this country, and had such a friendly feeling for us, that the declaration of war must have been a great blow to him. Jost, after speaking of Solms' many friends in England, adds, "Um so grösser war im August, 1914, sein Schmerz, als er sehen musste, dass auch solche Freunde dem deutschen Volk den Krieg erklärten,"—rather a pathetic expression of the German point of view.

Dr. Jongmans informs us that while Solms did not agree with the "stupidities" of certain German extremists, he was much annoyed at some English war publications depreciating German science and asserting the impossibility of future scientific collaboration. He was convinced that science must always be international. Dr. Lotsy writes: "He certainly was a good German, but in the best sense of the word."

Finally, the writer is permitted to quote a letter from Miss G. Lister, F.L.S., with which this notice may appropriately conclude: "I felt a great personality had passed away when I saw a few days ago in 'Nature' that Count Solms was dead. . . . . How well I remember his kindly behaviour towards us when my father and I came to Strasburg to look through De Bary's Myxos—the fine room we were given to work in, and his daily visits to see how we were getting on—always with a cigarette in his fingers, and the request 'Is it permitted?' before he smoked, and his planning a little excursion for us to the Black Forest to see the 'young green' of the beeches. Then the delightful visit he paid us at Highcliff—all past and gone! The blessed thing is to think the friendship can never be disturbed now, and the memory of his noble life remains."

Besides those names already mentioned, the writer is indebted to Prof. W. G. Farlow, Prof. A. G. Nathorst, and Prof. F. E. Weiss, F.R.S., for information and help.

D. H. S.

ROBERT BELL, 1841–1917.

Dr. Robert Bell died on June 18, 1917, at Rathwell, Manitoba, in the 77th year of his age. He was born in Toronto on June 3, 1841, and was a son of the Reverend Andrew Bell, of the Free Church of Scotland. He was educated at the Grammar School of the county of Prescott, Ontario, and subsequently at McGill University and the University of Edinburgh.

While still a boy of 15 years of age he joined the Geological Survey of Canada, under Sir William Logan, in the capacity of Junior Assistant, and was rapidly promoted in the service, where he remained—with but one short interval—for over fifty years, retiring as Chief Geologist and Acting Director in the year 1908.

He married Agnes, daughter of the late Alexander Smith, of Westbourne, Glasgow, and Auchentroig, Stirlingshire, and leaves one son and three daughters.

Practically nothing was known of the geology of Canada, and very little with reference to its geography, when Mr. William E. (afterwards Sir William) Logan was called upon to undertake the organisation of the Geological Survey of Canada, and, as its first Director, to carry out the mapping of this vast area and the examination of its mineral resources. Dr. Bell's work, as a member of Sir William Logan's staff, in these early years consisted therefore exclusively of geological reconnaissance and geographical exploration. Even in later years, and until his administrative work confined him to his office, Dr. Bell's work remained essentially of this character.

The exploratory traverses and track surveys which he carried out were chiefly within the area of the great Laurentian protaxis or "Canadian Shield," which forms such a striking feature, and which has played, and will continue to play, so important a rôle in shaping Canadian history and in influencing the character of the Canadian people. Dr. Bell's lines of exploration crossed and re-crossed this area from the Atlantic Coast to the Great Lakes on its western border, and from Lake Huron and Lake Superior on the south to the Arctic Sea, following the waterways of this great land. He also worked along the coast-line when acting as Geologist and Naturalist to the "Neptune" Expedition in 1884, and to the "Alert" Expedition in 1885, both of which carried out explorations in Hudson Bay and Hudson Straits; also on the "Diana" Expedition in 1897, when he surveyed the coast of Baffin Land, and was one of the first white men who penetrated to the great lakes in the interior of that immense island. Probably no man has ever traversed this great pre-Cambrian area so completely as did Dr. Bell.

The results of his work were published chiefly in the 'Annual Reports of the Geological Survey of Canada,' but he also contributed papers—some 200 in number—to leading scientific and technical journals in Great Britain, Canada, and the United States.

Dr. Bell's work on the Geological Survey of Canada was interrupted from 1863 to 1867. During these years he occupied the Chair of Chemistry and Natural Science in Queen's University, Kingston, Ontario, which Chair he resigned at the end of this period to resume his work on the staff of the Geological Survey. He was not only a geologist, but had also a good general knowledge of natural history, and his reports contain much information concerning the flora and fauna of the remote regions in which he worked. His travels in these wild northern lands also brought him into intimate relations and association with the Indians, who, with the exception of the employees of the Hudson Bay Company, were at that time their only inhabitants. His expeditions were carried out almost entirely in birch-bark canoes, with Indians or half-breeds as his guides and voyageurs. He thus acquired an extensive knowledge of Indian folk-lore and legendary story, and was so honoured and esteemed by the red-men that he was elected as a chief by the Algonquin Indians of Grand Lake.

In two papers read before the British Association for the Advancement of Science in 1881 and 1909 respectively, and also in a pamphlet entitled 'A New Route to Europe from the Interior of British North America,' published in Montreal in 1881, he advocated strongly the development of the Hudson Bay route, to which since that time so much attention has been directed.

Dr. Bell was the recipient of many academic degrees, and many honours were conferred upon him by learned societies. He received the degrees of D.Sc. (Cantab. and McGill), M.D., C.M. (McGill), LL.D. (Queen's). He was elected into the Royal Society in 1897. He received the Imperial Service Order in 1903. He was one of the Foundation Members of the Royal Society of Canada, and a Fellow of the Geological Society of London and of the sister Society in America, as well as of many other learned bodies. He was a member of the Geographical Board of the Dominion of Canada.

Dr. Bell was awarded the King's or Patron's Gold Medal of the Royal Geographical Society in 1906, and the Cullum Gold Medal of the American Geographical Society in the same year. His name will always be associated with the early exploration and the development of the Dominion of Canada.

F. D. A.

EDWARD HULL, 1829–1917.

The pursuit of geology seems eminently calculated to prolong life, doubtless due to the healthy open-air existence which its study involves. Many names of distinguished geologists who have passed away might be mentioned in support of this view, and now we have to add to the list that of Prof. Hull, who survived to his 89th year, having maintained his usual health and vigour to within a month of his death. He was the eldest son of the Rev. J. D. Hull, curate in charge of the parish of Antrim, where he was born on May 21, 1829. At the age of 12 he was sent to the School for the Sons of the Irish Clergy, then at Edgeworthstown, in County Longford. His life there was not happy, but after the transference of the school in 1843 to Lucan, near Dublin, and the appointment of a new headmaster, he appears to have enjoyed school life. It was his father's wish that he should become a clergyman of the Church of Ireland. With this object in view, he joined a class of students of the Irish language, conducted by the Professor of Irish of Trinity College, and gained a prize in this subject given by the University. About the same time a course of experimental lectures on hydrostatics, mechanics, and allied subjects, given at the school by a Dublin doctor, aroused his interest in natural science, and the idea of entering the Church was given up. Owing to the recent rise and progress of railway construction all over the country, there were, at that time, many openings for civil engineers, and it was therefore decided that he should be trained for that profession. He accordingly entered Trinity College, and at the end of four years not only obtained a diploma for civil engineering, but also graduated in Arts.

Among the subjects taught during the engineering course was that of geology. This brought him in contact with Prof. Oldham, F.R.S., Director of the Geological Survey of Ireland, whose lectures were delivered with such lucidity and attractiveness that, to use Hull's own words, "I found geology to be the subject that of all others captivated my mind." Failing to obtain immediate employment as a civil engineer, he was introduced by Oldham to Sir Henry de la Beche, who was then Director of the Geological Survey of Great Britain. His application for employment on the Survey, supported by Sir Roderick Murchison, with whom his family was distantly connected, was granted, and so, once again, his plans for a career were changed, for the third and last time.

Hull began work on the Geological Survey in Wales under Beete-Jukes, afterwards Professor, in 1850, and during the succeeding years took part in the mapping of a tract of country in the west of England, chiefly on Jurassic rocks. The results of this early work are recorded on the maps of the Geological Survey and in four descriptive "sheet-memoirs," of which he was either author or part author, published between 1851 and 1861. One of these

Edward Hull

memoirs, that on the 'Geology around Cheltenham,' illustrated with maps and sections and a plate of fossils by C. R. Bone, deserves special mention as an excellent example of combined geological and palæontological work. More money was expended upon these earlier memoirs of the Survey than on those of later date, which were too often treated very parsimoniously by the authorities.

Subsequently, Hull surveyed a large portion of the Lancashire coal-field with bordering areas, and this ground was described between 1860 and 1866 in five sheet memoirs from his own pen, with a sixth written in collaboration with the late Prof. A. H. Green. He also produced in 1869 a district memoir on the Triassic and Permian rocks of the Midland Counties, which remains the only general account of these rocks that has so far been published.

During the 17 years of his work in England and Wales much time was devoted to the coal-fields of Lancashire, Cheshire, and North Wales, and the knowledge thus acquired led him in 1861 to publish his first separate work on the coal-fields of Great Britain; a treatise dealing not only with their structure but also with the probable quantity of coal, both in the known coal-fields and beyond their visible limits, to a depth of 4000 feet from the surface.

Soon after the publication of this book, the great question of the duration of our coal supplies attracted public attention, and this has led to a second, third, fourth, and even fifth, edition; a striking proof of the need as well as the value and importance of this work of reference both geologically and commercially.

As a further result of his work on the Survey in England, Hull wrote several suggestive papers, including one of special interest, "On Isodiametric Lines as a Means of representing Sedimentary Clay and Sandy Strata, as distinguished from Calcareous Strata, with special reference to the Carboniferous Rocks of Britain" ('Quart. Journ. Geol. Soc.,' vol. 18, 1862). In it he points out that, where mechanical and organic sediments are associated in any formation, one group increases in thickness as the other diminishes. In order to illustrate this point he gives a map of Great Britain on which the thicknesses of the two groups of sediments in the Carboniferous rocks are indicated by a series of lines drawn through the places where these thicknesses are known or assumed to have been equal. The map brings out in a most striking manner the contrast, so far as distribution is concerned, between the two types of sediment, and indicates the general directions from which the mechanical sediments were derived. Subsequent research during the past fifty years has not seriously modified the conclusions at which he then arrived.

In 1855 Hull was elected a Fellow of the Geological Society of London, and in 1867 a Fellow of the Royal Society. In this latter year also he was transferred to the Geological Survey of Scotland as District Surveyor. After a short term of two years in Scotland (1867–8) he was appointed Director of the Irish branch of the Survey, a post rendered vacant by the

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untimely death of Prof. J. Beete-Jukes. While in Dublin he not only served as Director of the Geological Survey, but also fulfilled the duties of Professor of Geology in the Royal College of Science, an appointment which had been held by Oldham and by Beete-Jukes. The post of Director of the Irish Survey was not without its difficulties, and it stands to Hull's credit that he carried on the labours of his predecessors with such success that by the time of his retirement, in 1891, not only were all the sheets of the 1-inch Geological Map of Ireland published, but each sheet was accompanied by a descriptive memoir. One wishes the same could be said of England and Scotland. Either as sole or part author, Hull's name appears on the title-pages of nine of these memoirs, chiefly relating to the northern part of the island.

In 1871 the first Royal Commission issued their 'Report on the Coal Supply of Great Britain and Ireland,' to which Prof. Hull contributed much valuable information, and, owing to the death of Prof. J. Beete-Jukes, who was one of the Commissioners, he prepared, or edited, the final report on Ireland. Thirty years later a second Royal Coal Commission was appointed, on which Hull served as a member, devoting five years to this important subject, 1901–5. In February, 1890, the Council of the Geological Society awarded the Murchison Medal to him, and in the following year he retired from the Survey, having been in office under four successive Directors General: de la Beche, Murchison, Ramsay, and Geikie, names which will ever be associated with the history of geological science.

But his activity did not cease with his retirement from official work. First as Secretary and later as President of the Victoria Institute, he contributed many papers to their publications. He also contributed to the 'Geographical Journal' and to the 'Geological Magazine,' and published, independently, a large atlas and memoirs dealing with the 'Sub-oceanic Physiography of the North Atlantic Ocean,' a subject in which he became much interested during the later years of his life. The complete list of his published memoirs, books, and papers contains more than 250 entries, many of which relate to other subjects than pure geology.

In 1883, on the recommendation of General Sir Charles Wilson, Hull was appointed as leader of an expedition, despatched by the Committee of the Palestine Exploration Fund, for the purpose of carrying out a topographical and geological survey of portions of Arabia Petræa and Palestine. To the late Lord Kitchener (then Captain), with the assistance of a staff from England and Egypt, was entrusted the topographical work. The expedition travelled from Cairo through the Peninsula of Sinai to the Dead Sea by the Arabah valley, crossing Southern Palestine to Gaza and visiting Jerusalem. In his report he calls attention to the great fracture in the earth's crust extending northwards from the Gulf of Akaba to the Dead Sea and along the valley of the Jordan. The narrative of this most successful expedition was published in 1884 by the Committee of the Palestine Exploration Fund in a work entitled 'Mount Seir, Sinai, and Western Palestine.'

Keith Lucas.

Hull's kindly spirit and geniality will long be remembered, especially by those who took part in the friendly gatherings of the Royal Dublin Geological Society and the Irish Microscopical Society. His long career extended from the days of de la Beche, Sedgwick, Murchison, Lyell, and Prestwich through those of Ramsay, Forbes, and Huxley down to the present day, from the youthful period of our science to that of its full grown manhood as we see it now. In all these surroundings of men and of progress, Edward Hull moved and "played his part," and did his best to advance geological knowledge, both in his full period of official life on the Survey and in the later years of his activity as "a geologist at large," always striving to contribute his quota to the "advancement of natural knowledge," for which, as a Fellow of this Society, he was elected 50 years ago.

H. W

KEITH LUCAS, 1879–1916.

PART I.

KEITH LUCAS was born March 8, 1879, and was killed on October 5, 1916, when he was 37 years old. His death was caused by a collision with another aeroplane in mid-air, when flying over Salisbury Plain, and was instantaneous.

He was the son of Francis Robert Lucas and grandson of Ralph Willett Lucas, a Lieutenant in the Royal Artillery who fought in the Battle of Waterloo. His father, Francis Lucas, went as engineering pupil to the Telegraph Construction and Maintenance Company, East Greenwich, when he was 15 years of age and was one of the young engineers chosen to go out in the S.S. "Great Eastern" when the first Atlantic cable was successfully laid. He afterwards went in her on every voyage. After this he was made manager of the works at Greenwich, but continued to go to sea and lay cables until he had laid the Pacific or "All Red Cable" round the world. He then gave up the work at sea and became Managing Director of the Company. During his life at the works he was continually inventing and improving the machinery for cable manufacture and cable laying. Many of his inventions have been adopted by the Admiralty as well as by cable companies.

Keith Lucas' great-grandfather on his mother's side, Edward Riddle, and his grandfather, John Riddle, were both good mathematicians, both were Fellows of the Royal Astronomical Society, and both had great reputations as teachers of navigation and nautical astronomy.* Lucas inherited from his ancestors his scientific interests and ability, his power of designing new apparatus, and his engineering capacity, as well as his great manual dexterity.

From the Rev. T. Oldham's preparatory school at Blackheath, he gained a Classical Scholarship at Rugby in 1893. He was in the Volunteers at Rugby, and in the Shooting Eight for four years; and was captain of it in 1897–8. He was head of the School House before he left to come up to Trinity College, Cambridge, with a Minor Scholarship in Classics.

His interest in Engineering and Science was encouraged at home, where he learnt the use of many tools. His work was from the first neat and practical. His mastery of the design of new instruments, coupled with his capacity of making what he wanted, gave him great power in the art of experiment in such dissimilar sciences as Physiology and Aeronautics.

At Cambridge he devoted himself to Science, and took a First Class in the Natural Sciences Tripos, Part I, 1901. After the Tripos, he felt the strain of doing so much work in a new direction, and at this time his old school friend, Charles Powell, was killed in the Boer War. Lucas felt his death acutely. He gave up working for the second part of the Natural Sciences Tripos and went to New Zealand for the sake of rest and change. There, he measured the depth of some lakes reported as bottomless, and when he returned to England he published the paper, "A Bathymetrical Survey of the Lakes of New Zealand," in the 'Geographical Journal,' May and June, 1904. His father's experience in deep-sea sounding and cable laying was naturally helpful to him. This was the beginning of his original work. The advances he made in Physiology are described in a separate Memoir, and were not interrupted till the war induced him to devote his abilities to Aeronautics.

He was elected a Fellow of Trinity in 1904 and in the same year gained the Walsingham Medal and the Gedge Prize. He was appointed Lecturer in the College in Natural Sciences and took the Degree of D.Sc. in 1911. He was elected a Fellow of the Royal Society in 1913, having already delivered the Croonian Lecture in 1912. He also gave the Page May Memorial Lectures in connection with the Institute of Physiology, University College, London, in 1914.

He felt that all should take their full share in the work connected with the administration of the University and College business. He was on the Council of his College for some years, spoke little, but always to the point, and took his full share in decisions, and his opinion was highly valued. When the new Physiological Laboratory at Cambridge was being planned, and later, before it was occupied, he did much to make the arrangements satisfactory and efficient.

Keith Lucas was keenly interested in and enjoyed his work as a teacher, both in his College classes and in his University lectures and demonstrations. He felt the importance of this work, and, with his knowledge of the difficulties of the subject and his power of clear explanation, it was natural that he should be a most successful teacher. The work he had himself done in Physiology, and his hope for far greater advances, both by his own work and the work of others, inspired in his pupils enthusiasm for further investigations.

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* 'Dictionary of National Biography' and 'Monthly Notices of the Royal Astronomical Society,' February, 1855; 'Monthly Notices of the Royal Astronomical Society,' February, 1863.

In 1909 Keith Lucas married Alys, daughter of the Rev. C. E. Hubbard. He leaves three sons. He became a Director of the Cambridge Scientific Instrument Company in 1906, and only resigned in 1914, when he joined the Royal Aircraft Factory. During this time he designed many instruments both for teaching and research.

Even when he was in New Zealand, and before he had begun his original scientific work at Cambridge, and had so much to do with the design of scientific instruments, he thought that the great flaw in instrument making was that there had to be much perfect and expensive workmanship to make up for faults in design. He agreed with the definition of a well designed instrument as one which worked smoothly and well, and gave accurate results when the rubbing surface became worn or the parts damaged, and even if badly made. In his designs, each moving piece was allowed only the requisite freedom to move in the proper manner, and was guided only at the correct number of points. The importance of these considerations was long ago pointed out by Clerk Maxwell and Lord Kelvin, and generally leads to a good design as defined above.

In his work with the capillary electrometer, he had to analyse a great number of photographic curves; to do this he designed an instrument which saved a great deal of time, and gave results with remarkable accuracy. In connection with this work, he designed a rapid and trustworthy method of drawing fine capillary glass tubes. He also designed a photographic time-marker on the principle of the Einthoven string galvanometer, in which the self-induction and inertia were much reduced, and the time-lag was extremely small. Among other instruments, he designed apparatus for breaking two electric contacts at short intervals apart, and many useful instruments for teaching.

Like so many of the best experimenters, he made with his own hands much of the apparatus he wanted for research, and his skill allowed him to use the simplest means to get good results. Some of the more elaborate instruments, however, were made by the Cambridge Scientific Instrument Company, and he often discussed the designs with me, as Chairman of the Company and as his personal friend. This was always a pleasure; he knew what the instrument should do and how the parts should be made, and his quickness in realising the difficulties, and in seeing improvements in a mechanical design, was most striking. Sometimes I felt proud that I was able to improve the work of such a master of the art of design. If this happened to be the case, his quickness and fair-mindedness made him realise the fact at once. If it were not so, he very soon proved that he was right. All he wanted was to get the best out of whatever work he was doing; it was the good quality of the work he cared for, not any personal credit in connection with it. This tendency to think of his work and not of himself was appreciated at the Royal Aircraft Factory, and is described in the 'Court Journal' of October 20, 1916, where the writer adds: "I wish there were more like him in that way."

When the war broke out he at once became a volunteer, and did useful work in patrolling the road to Newmarket on a motor bicycle. He then passed the medical examination for the Honourable Artillery Company, and was about to enlist as a private in the infantry battalion. Just then I happened to meet him, and realised at once that his value to the country would be far greater if he worked at the Royal Aircraft Factory, and a telephone conversation with Colonel O'Gorman, Superintendent of the Royal Aircraft Factory, the same day, removed all doubt, and he began work at once.

His scientific training and attainments, his knowledge of the manufacture of scientific instruments, and his remarkable powers of design and research, enabled him to do most valuable work there. Colonel O'Gorman, C.B., Keith Lucas' commanding officer, and Superintendent of the Royal Aircraft Factory during the two years he worked there, writes:—

"Shortly after the outbreak of war, amongst those who flocked to the colours was Keith Lucas. He was a straight, well-knit man, slender, but active, with a body exceptionally finely controlled by an energetic mind. He was young for the manifold scientific honours which distinguished him, but he was so far from over-appreciating himself that at one time he was about to enlist as a private. But, instead of this, he was able to use his rare scientific abilities in improving military aeronautics.

"By a lucky chance, I had the opportunity of giving him work at the Royal Aircraft Factory after he had passed the medical examination for the Honourable Artillery Company.

"I had previously met him when staying in Cambridge, and had heard him discuss questions of mechanical design. This was enough to leave no doubt of his utility, and I seized the suggestion of his joining the Royal Aircraft Factory.

"For Lucas this meant leaving his home at Cambridge, and giving up his original scientific work in Physiology, which was the dominant and all-absorbing interest of his life.

"He arrived at the Royal Aircraft Factory on September 4, 1914, and after living at Fleet for some months, took up his residence in the little wooden hut, 12 feet by 10 feet, which was the only possible means of being housed in the crowded neighbourhood, where workpeople were sleeping as many as 11 in a six-roomed cottage, or using in pairs, for alternate day and night work, the same beds. To be near his work was essential, as in summer he was often flying at dawn.

"He was entrusted with one problem after another in rapid succession, while, simultaneously with this, he held himself open to be consulted, and was constantly consulted, on numerous problems.

"The Experimental Research Department was evidently the place where his abilities would have scope, and there he took up his work. This was the department which had so lately been presided over by Edward Busk, a distinguished graduate of King's College, Cambridge, with whom were associated a number of other Cambridge men, with whom Lucas enjoyed working.

"In breaking new ground of the kind to be dealt with in this department many of the steps meant making measurements of quantities which had never before been measured, and in this he must have found a link with the analogous difficulties in his own study of Physiology. Methods had to be evolved, and instruments to be designed and made. Here Lucas excelled, and in the instrument shop, under Mr. F. Short, he was welcomed and honoured by staff and mechanics alike.

"It chanced that the problem of how to make an accurate sight for dropping bombs from aeroplanes was under consideration shortly after Lucas came. He worked at this, and the seed has been sown which will greatly improve the aeroplane as an offensive weapon.

"On the way to his solution, by means of the gyroscope, he evolved his 'space damped pendulum,' which is in large measure free from the effect of the movements and oscillations which the aeroplane imposes on everything within it. This was a simple device, which avoided the complexities attendant upon the use of gyrostats, and one of its practical outcomes was a new instrument, an aeroplane level, of considerable use in a number of further experiments on aeroplane flight.

"The trend of all this work indicated how necessary it was to obtain an autographic record of the movements, of roll, pitch, and yaw of an aeroplane, both when the pilot abandoned all control, and also when the pilot exercised his utmost vigilance in correcting all deviations. This Lucas was asked to do; he completed a method outlined for this purpose by Mr. Busk, and simplified it. He worked hard, rising day after day at four in the morning for flights when the air was at its stillest and sun low, and, in conjunction with Captain Mayo and Major Goodden, eventually produced a beautiful series of curves of motion which were sent in to the Advisory Committee on Aeronautics, and were received with marked approval.

"With these data, he now knew what classes of erratic motions he had to deal with, when either the flyer or the wind gusts interposed to upset or alter the aim taken with his bomb sight.

"By this time he had decided that the use of the more complex gyrostat could not well be evaded in favour of his simpler scheme of the 'space damped pendulum.' He adopted a suggestion of Major Hopkinson, F.R.S., who had also been for some months a member of the Royal Aircraft Factory staff, and made an improved bomb sight.

"A new and somewhat startling difficulty had been found in connection with aeroplane compasses. Fliers, lost in the fog or cloud and persisting in what they thought was a careful compass course, would find themselves facing in a direction opposite to that in which they believed they were—they would come out of a cloud where they went in without having deviated from a compass course which should have taken them straight through it. This was given to Lucas to solve. He first found the causes of the erroneous indications, and then made a compass in which they were greatly reduced; and his 'space damped pendulum' inspired one part of the remedy. A portion is due to Mr. H. Darwin, F.R.S.

"Lucas was a master of clear and lucid exposition, so that, though he was not always easy to draw into any long dissertation, if he once decided to state a case, there was no loophole for misunderstanding him.

"On the formation of a Territorial Unit of the Royal Flying Corps recruited from the employees of the Royal Aircraft Factory, Lucas was one of the first to be approached by myself, as officer in command, with a view to his taking command of a Park—roughly, 400 men. He willingly consented to take the additional work and responsibility. He was gazetted Captain on December 1, 1916, and appointed to the command of No. 3 Park. He threw himself whole-heartedly into his military duties, and this was soon reflected in the discipline of his command.

"He was essentially a popular officer, and this in a military sense. His men had implicit confidence in his ability to lead them, and no greater tribute is needed.

"I invited him to be the first mess president of the officers' mess, and he retained this office till his death. He had a good influence over the younger officers, and his loss will be greatly felt.

"He was a regular attendant at the Commanding Officer's lectures, and in his own lectures on technical subjects no officer held the attention of his audience on seemingly dry subjects so perfectly as Lucas. His clear style and unusual form of wit made his subject interesting to the latest promoted non-commissioned officer.

"His request to be allowed to learn to fly was granted, and he went to the Central Flying School, where he acquired the art remarkably quickly. He never had a mishap until the fatal collision in the air, when the air-screw of the other aeroplane struck him. He was undoubtedly killed instantly."

Colonel O'Gorman has pointed out the value of Lucas' work on aeroplane compasses. An error, which we will call "the turning error," had often been noticed on aeroplanes. When an aeroplane turned to the right or the left the compass did not indicate the magnetic north correctly. He not only found the cause of this error but designed and made a compass which reduced the error to a great extent. It was, however, a disappointment to him that he was not able to eliminate the error completely. The first difficulty to be overcome was to find out why the error was capricious; sometimes it showed itself and sometimes it did not, and it became clear that although it depended on the rate of the turn, it also depended on something else. After much flying and observation of a compass in the air, he found that this error was very great if a deviation was made when the direction of flight was towards the north. In this case the compass needle was so far carried round with the aeroplane, when on a turn, that the flier might think he was flying in a straight line, although he was turning somewhat rapidly. In a cloud, or at night when there were no visible objects to act as guides, this was a great danger. If the aeroplane was flying in a southerly direction the compass needle turned in the opposite direction, and the flier would get an exaggerated estimate of his rate of turning. As the flier's object is to fly straight through a cloud this would not matter.

The abnormal behaviour of the standard compass in the air was utterly unexpected, and the value of the discovery was great. In order to remedy this defect, which was found in all existing compasses, much experimental work had to be done in the air; he formed theories of the cause of the error, tested them in the air, and after eliminating those which proved wrong, at last found the true cause.

The magnetic forces act on the poles of the magnet in the direction of the dip, and tend to rotate it in a vertical plane. When the aeroplane is flying straight this tendency is balanced by displacing the centre of gravity towards the south pole, in order to keep the magnet horizontal. But when the aeroplane is turning the apparent direction of gravity is no longer the true vertical; the magnetic forces, however, still act in a vertical plane, as before, and this change of conditions produces the turning error.

The compass that Lucas made was a great improvement on the existing patterns. Its special features are the combination of the antivibration mounting; the spherical bowl to contain the liquid; a magnetic system small in relation to the size of the bowl, with a long period of vibration; graduations on a short cylinder instead of a disc; and the inverted pivot.

Unknown to Lucas, most or all of these features had been tried before for marine compasses; but they are not called for at sea, owing to the relatively slow speed of ships, and had been long forgotten. By his work he brought them into use for aeroplane compasses, and they are an important life-saving factor.

One of the reasons why vibration causes errors in compasses was pointed out by Mr. A. Mallock to the Advisory Committee for Aeronautics, and it fell to me to be of some little assistance to Lucas with regard to this error. Theoretical considerations showed that this vibration error would be reduced by inverting the usual arrangement in compasses in which an agate cup is carried by the card and rests on a needle-point fixed to the compass bowl. If the needle-point is fixed to the magnetic system and the agate cup is supported by the compass bowl the vibration errors were reduced. Lucas looked into the theory of the vibration error, confirmed the experiments I had made, and adopted the inverted point support, and it is a great satisfaction to me to have been of some use in this improvement.

Many of Keith Lucas' friends heard, with regret, that he was learning to fly. In addition to their personal affection they felt the possible loss to science owing to the risk he was running; they also thought that the advances he was making in aeronautics were so important that no chance of interruption by an accident should be taken. But it was questionable whether the risk was increased. Before he learnt to fly he had been in very many flights on an aeroplane as a passenger when he was experimenting with various instruments, and for this work it was essential that he should be a passenger. When flying as a passenger an accident might happen through want of skill of the pilot; when he was a pilot his own want of skill might cause an accident, but those that knew him felt sure that when he had once learnt to fly, he would have far more than the average skill in manipulation of an aeroplane, requiring as it does a clear cool judgment and rapid co-ordination of muscles and brain. He thought he could do his work better by becoming a pilot, and improve the technical part of the branch of the service to which be belonged, and he was right.

Flying as a passenger gave him great pleasure, even on the first occasion. But the pleasure in his first flight alone—his instructor left behind—was far greater still, and he met his death swiftly and suddenly in the open air doing the work he loved. He was buried in the Military Cemetery at Aldershot.

HORACE DARWIN.

PART II.

Although the physiology of muscle and nerve, and the nature of the excitatory process which passes along such tissues in the form of a wave from a stimulated point, had been subjected to investigation by a large number of workers, it is remarkable how little advance had been made since the time of Helmholtz and Du Bois-Reymond. An occasional fact of importance was discovered from time to time, but it was not until Keith Lucas commenced his systematic study of the process in 1903 that any rapid progress took place. In his first paper, which was devoted to the question of the effect of tension on the duration of muscular contraction, we find from the outset how great a part the design of appropriate and accurate instrumental aid was to play in the elucidation of the various problems attacked. It was by the elimination of the inertia of recording levers by the use of a photographic method that it was shown that increase of tension, within limits, results in a lengthening of the period of contraction. This fact, at a later date, was destined to play an important part in the theory of muscular contraction.

Gotch had already obtained results which indicated that the different degrees of contraction which a muscle is able to exert were due to the varying number of individual fibres at work, and not to the capacity of each fibre to contract otherwise than to the maximal extent within its power at the time. Keith Lucas brought forward convincing evidence that Gotch's contention was correct, in that the number of degrees of contractile stress possible for a muscle to manifest is not greater than the number of nerve fibres supplied to it. In a subsequent paper, description is given of experiments on a muscle whose nerve contains only eight or nine fibres. In this case, the nerve itself was stimulated. Thus the contraction of voluntary muscle was brought into line with that of the heart and Bowditch's "all or nothing" law shown to apply. Still later, Adrian, a pupil of Keith Lucas, was able to extend the law to the nerve fibre itself, by the use of an ingenious method to be referred to below.

The next step in the theory of contraction was to show that the wave does not change in magnitude during its passage, so far as normal muscle is concerned, although it may suffer diminution in fatigued muscle.

An important series of papers claims our attention at this stage, a series which may be said to have their starting point in the observations of Waller that the amount of energy required to stimulate a nerve varies with the rate at which this energy is applied. Different nerves have a different "characteristic," due to the natural rate of movement possessed by some constituent, which rate controls the effective taking up of the incident energy. Keith Lucas' first experiments were made, as were those of Waller, by the use of condensers of adjustable capacity, charged to different potentials. Subsequently, it was found better to use as index the potential required with currents of varying durations, a factor related to the former in a definite way, since the energy is expressed by v^2t, where v is the potential and t the duration of a current. Similar results were obtained with a simple apparatus designed to vary the rate of increase of an applied current. The methods described were utilised in the analysis of complex excitable systems, such as the sartorius muscle with its nerve. It was found that the muscle has two distinct optimal rates of incidence of energy, one of a very much greater magnitude than the other. This statement still held after sufficient curare had been given to abolish the effect of stimulation of the nerve trunk, so that there must be some additional excitable substance situated between the nerve and the muscle. By testing a part of the muscle free from nerve endings, it was found that the low rate belonged to the muscle fibre itself. The nerve trunk was found to have an optimal rate rather higher than that of muscle, while the intermediate substance had an extremely high rate. The form of the curve expressing the time-course of the relationship between duration of current and potential required to excite, as the muscle changes after excision, was shown to be altered by the presence or absence of calcium. This fact was brought into relationship with Nernst's theory of excitation, to which more attention was given later.

Another property of excitable tissues which is connected with the time-factor in question is the summation of two stimuli, each just below effective strength. If the excitatory process set up by the first stimulus has not disappeared when the second arrives, there is summation. So that the fact depends on the rate of subsidence, or, in the terms of Nernst's theory, on the rate at which the concentration of ions brought about by the exciting current is again dissipated by diffusion. A further outcome of this point of view was the analysis of various excitable tissues in the light of A. V. Hill's modification of Nernst's theory, in which account is taken of the distance between the membranes at which the ions concerned are supposed to be concentrated and of the diffusion constants of these ions. The time-factor turned out to be in reality conditioned by these two components of the expression deduced by Hill. An interesting point, as yet not explained, is that the rates of movement of the ions in different excitable substances differ more than those of the ordinary inorganic ions known to be present.

Since the rate of the excitatory process should be increased by rise of temperature, it was natural to bring the different effects of temperature on the apparent excitability of muscle and nerve to constant and induced currents into connection with their optimal rates of incidence of energy. The explanation was found to consist in two opposite effects of fall of temperature. A fall of temperature means, on the one hand, a greater ease of the production of the necessary concentration of ions, owing to the decrease of opposing diffusion, while, on the other hand, the actual initiation of the propagated disturbance is more difficult. The resultant effect varies according to the duration of the current required to excite a particular tissue.

The temperature coefficient of the rate of conduction in nerve was measured by Keith Lucas, using an extremely accurate method. It was found to be 1·79 for the 10 degrees between 8° and 18° C.

For further progress it was necessary to make use of the electrical change in excitable tissues as indicating the excitatory process. For this purpose an improved form of capillary electrometer was invented, together with apparatus for measuring the curves for the purpose of analysis. It was first shown that the temperature coefficient of the rate of conduction in excitable tissue is the same as that of the time of development of the electrical disturbance. Hence there is no difficulty in taking this latter as the basis of propagation of the excitatory state, although no proof of their identity is given thereby.

The next series of papers are devoted to the refractory period which follows an effective stimulus. It was shown that the time which elapses before an electrical change shows itself, when it is due to a second stimulus following a previous one, is constant, although the time after the end of the refractory period at which the second stimulus is given may vary considerably. This fact suggested the name "irresponsive period" for the interval between an electric response and the earliest possible succeeding one. This delay occurs also in ventricular muscle, and is due entirely to a modification of the tissue by the preceding propagated disturbance, and not to any direct effect of the current used for stimulation. In conjunction with his pupil, Bramwell, Keith Lucas next showed that the decrease of excitability after an effective stimulus is due to the passage of the propagated disturbance itself. This refractory period is, in fact, independent of whether the two stimuli fall on the same point or on different points.

A peculiar phenomenon, known as Wedensky's inhibition, also found its explanation in Keith Lucas' work. It was shown to be due to a resistance to conduction in the nerve greater than normal, and the possibility of similar conditions in the nerve centres was discussed as a probable basis of some inhibitory phenomena.

A further step was taken, in conjunction with Adrian, towards the analysis of the process of stimulation, in that this was shown to consist of two stages, a purely local effect and a propagated effect. The former may be present, although insufficient to set in motion a propagated disturbance, and the fact that it does not immediately disappear renders possible summation of stimuli, each in itself an inadequate one. An important method was developed for the estimation of the magnitude of a propagated disturbance, as referred to above. This consisted in determining the distance travelled through a region in which it is progressively diminished in magnitude, that is, a region of decrement, such as is produced by anæsthetics. This method was afterwards applied by Keith Lucas himself to the decision of the important and disputed question as to the possibility of distinguishing between conductibility and excitability in nerve, a distinction which, it had been stated, could be made out by the use of alcohol as a narcotic. The two factors were shown in reality to disappear together, and this disappearance to be due to the same cause, namely, increased difficulty in setting up a propagated disturbance. It was also found that, when such a nerve impulse is set up by a strong stimulus in the "relative" refractory period, it is smaller in magnitude than the normal one, produced by a stimulus outside the refractory period.

The Croonian Lecture was given by Keith Lucas in 1912, and was devoted in part to a discussion of the work referred to in the preceding pages, and to the investigations of other workers on questions related to it. The Lecture concluded with a more detailed description of the modified form of Nernst's theory of excitation, and with criticism and suggestions concerning it.

In a valuable paper on "Summation in the Claw of the Crayfish," published in the 'Journal of Physiology' after the author's death, some of the facts previously discovered are made use of to elucidate the complex problems of this interesting neuro-muscular mechanism. By application of the law governing the relation of current strength to the time of closure required to stimulate, it was found that there are two sets of nerve fibres in the nerve to the claw. One of these is responsible for a slow prolonged form of contraction, the other for a brief quick twitch. It was also found that the type of summation described by Richet was due to the fact that the first stimulus, although sending an impulse along the fibre, fails to cause contraction because it has been reduced in intensity by having to pass through some area of decrement on its course, probably at the synapse of the nerve fibre with the muscle fibre. A second stimulus, however, applied to the nerve during the period of increased excitability following the previous stimulus, produces a disturbance of sufficient magnitude to pass the obstruction, and contraction of the muscle results. This period of super-normal excitability, succeeding the relatively refractory phase, is more strongly developed in the crayfish than in the frog.

In a footnote to this paper, reference is made to some experiments on the phenomena of inhibition and of tonus shown by the muscles of the crayfish claw. It is much to be hoped that notes of these experiments may be found sufficiently detailed to enable them to be published.

Early in 1914 Keith Lucas gave the Page May Lectures at University College, London, choosing as his subject the phenomena of conduction in nerve. At the outbreak of war, the greater part of these Lectures had been written out for publication as one of the monographs of Prof. Starling's series. After the author's death, the manuscript was revised and completed by Captain Adrian, and the Lectures, which give an excellent summary of the knowledge gained in this field, for the most part by the work of the author and his pupils, were published towards the end of 1917.

It will be seen how great a loss physiological science has sustained in the death of so ingenious and talented a worker at so early a stage of his work. Many further and fundamental advances would undoubtedly have been made if only these researches could have been continued.

W. M. Bayliss.

H G Plimmer.

HENRY GEORGE PLIMMER, 1856–1918.

Henry George Plimmer was born in 1856, and was the son of a doctor enjoying a good practice in Wiltshire. He always believed that it was from his father that he inherited his love of music, but the country doctor did not transmit to his son that love of sport of all kinds which was so firmly implanted in himself, and unfortunately helped to account for the circumstance that on his death, which occurred when the boy was about nine years of age, he left his family with but slender resources. It was owing to this that young Plimmer entered business in 1870, being engaged as a clerk in a company with which his maternal uncle was concerned. These do not seem to have been very happy days, for the youth was dissatisfied with his outlook and prospects, and felt he was destined for other things. His musical talent, which grew with his growth, was developing rapidly at this period, and he used to tell of the joy with which he had access to the church organ, and of the musical festivals at Birmingham and elsewhere that he attended. He was also already laying the foundations of that wide knowledge of literature which distinguished him in after life, and he numbered Ruskin among his correspondents even at that time.

He finally determined to abandon a business career and to enter the medical profession, and his chance came as the result of a letter to Dr. J. H. Galton, a man who had formerly been, for several years, his father's assistant. In 1878 he came to London as an "unqualified assistant" to Dr. Galton, and thus got his foot on the lower rungs of the medical ladder.

The work was hard, but he had plenty of grit, and by dint of strenuous exertions on his own part he became qualified in 1882 as L.S.A. and in 1883 as M.R.C.S.

In the meantime he had not neglected his other opportunities. He had acquired a good knowledge of French and German, and had already visited both Germany and Belgium. In 1885 he entered into partnership with Drs. Turner and Galton, but in 1892 he retired from practice in order that he might devote himself to Bacteriology and kindred research. In October, 1892, he published an account of some admirable observations he had made on Cancer, and first described those cell inclusions which have come to be known as "Plimmer's bodies." These researches brought him into immediate contact with Armand Ruffer, who suggested that they should work together in the laboratories of the College of Surgeons and Physicians. To this proposal Plimmer agreed, and their association continued almost till 1894, when Plimmer was appointed as Pathologist to the Cancer Hospital. The friendship between the two men lasted until the death of Ruffer, who perished at sea during the war. A felicitous tribute to Ruffer's memory and work appeared in 'Nature' (1917), written by his friend, who was himself so soon to pass away.

In 1898 he became Bacteriologist to St. Mary's Hospital, and in the next year was appointed Pathologist and Lecturer on Pathology in the same institution. He resigned his appointments at St. Mary's in 1902, and undertook the direction of the Cancer Laboratories at the Lister Institute.

Meanwhile he had become interested in Trypanosomes, the organisms which produce Sleeping Sickness. In this connection his unrivalled skill in microscopic technique stood him in good stead. He was the author of a number of papers on this subject, and he became an active member of the Tropical Diseases Committee of the Royal Society.

In 1907 he extended the sphere of his pathological work by assuming the duties of Pathologist to the Zoological Gardens, which afforded him additional opportunities of gaining valuable experience. He held this appointment for ten years, finally resigning it as a protest against certain administrative changes of which he felt himself unable to approve. Plimmer communicated the results of his investigations in a considerable number of papers which appeared in various medical and scientific periodicals both at home and abroad, his work for the most part relating to Cancer, Trypanosomes, and kindred subjects. He was elected to the Fellowship of the Royal Society in 1910, and always displayed a keen interest in everything that concerned its welfare. Amongst the valuable services he rendered to the Society, one is especially deserving of mention. He undertook and carried out the overhauling, sorting, and cataloguing of the valuable engravings in the possession of the Society, a work of no small magnitude. It was fortunate, indeed, that the task, which to him was a labour of love, should have fallen into such judicious and capable hands. It was largely owing to his energy, and to his wide knowledge of matters pertaining to art, that so magnificent a series of portraits have been made accessible, with a convenient index, to the Fellows.

A Chair of Comparative Pathology was founded for a term of years at the Imperial College of Science and Technology by an anonymous donor, and Plimmer was appointed to fill it. He held this post until his untimely death in June, 1918. During his tenure of this Chair he delivered a remarkable series of lectures on Immunity, a branch of research in which he had long been keenly interested. He had been on terms of personal intimacy with the chief of the great Continental workers in this subject, from Pasteur onwards, and his brilliant exposition will long be remembered by those who were privileged to listen to him. As a teacher he was remarkably successful. His unusual cast of mind, his wide and varied knowledge, together with a singular personal charm, combined to exert a strong influence on the students who were so fortunate as to come into contact with him. Behind the professor there was always the kindly sympathetic personality of the man himself, who was richly endowed with wisdom in those things which really matter in life.

He had, for some years before his death, taken a prominent part in the administration of various learned societies. He was President of the Royal Microscopical Society in 1911–12, and he served on many scientific committees both at home and abroad. On the outbreak of the war he at once placed his services at the disposal of the country, and was actively engaged in various medical and scientific enquiries arising out of the new conditions imposed by the war. He was a member of the Tetanus and of the Trench Fever Committees, and was strenuously engaged on researches connected with the latter when he was stricken with mortal illness. He held on to his work as long as it was humanly possible for him to do so, and it was a source of great grief to him during his last days that he should not have been spared to bring his investigations to the end, which seemed already in sight.

In 1887 Plimmer married Helena, widow of Alfred Aders, of Manchester. He owed much to the stimulating devotion of his wife, who ever displayed an active interest in all the many-sided activities of his life, for he was not a scientific man only, but possessed an unusually extensive knowledge of art, literature, and especially of music. As a musician, indeed, he was in the very front rank in respect of his powers both of interpretation and execution. But his intimate friends, whether scientific or otherwise, perhaps will think of him most of all as a loyal and dear friend never to be forgotten, and one of whom it may truly be said, in the words of the poet whom he loved so well:—

Non omnis moriar, multaque pars mei
Vitabit Libitinam. . . .

J. B. F.

ALFRED MERLE NORMAN, 1831–1918.

The Rev. Canon Norman, M.A., D.C.L., Hon. LL.D. (St. Andrews), F.R.S. F.L.S., began his long and energetic career on August 29, 1831, at Exeter peacefully closing it at Berkhamsted, October 26, 1918.

Of his routine education at Winchester and at Oxford, where he took his degree from Christ Church, there is nothing special to record, though it is of interest to know that he studied Entomology at school, and the Mollusca of Oxfordshire while at college, and that in still earlier years his attention had been directed to Botany by his elder brother, who subsequently met an untimely death when Chief Justice of Bengal.

The interval of two years before his ordination at the age of 25, was spent at the island of Cumbrae, and it was probably this that stamped upon his future life a predominant, though not exclusive interest in Marine Zoology. For some years, indeed, his working hours must have been well filled with the business of his profession. This he never neglected, although in 1866 by becoming a rector he had thenceforth more the control of his own time. In his coal-mining parish of Burnmoor there was opportunity for interesting experiences, of which a sample is worth giving. Among the miners of his congregation he asked an influential friend whether he could not induce a particularly rough-looking mate to attend the services of the church. The appeal was successful, and the recruit, though unable to read, was seen to follow what he heard with earnest attention. Unfortunately, some time later the devotee appeared in a very disordered condition, which his neighbours attributed to his having had a drunken quarrel with his wife. Norman expostulated with him and the man expressed sorrow at what had happened. In time it turned out that it was the wife who had been drunken and inflicted the damage, which the husband was too chivalrous to assign to its true cause. Later on this good fellow was brought to his death-bed by a colliery explosion, and his dying whisper was: "I waited patiently for the Lord, and He inclined unto me, and heard my calling. He brought me also out of the horrible pit, out of the mire and clay, and set my feet upon the rock, and ordered my goings. And He hath put a new song in my mouth, even a thanksgiving unto our God."

On the scientific side of his avocations we find Norman associated with Gwyn Jeffreys, Professor McIntosh, H. B. Brady, G. S. Brady, Hancock, Spence Bate, and other naturalists of repute, not in holiday rambles, but in resolute and sometimes very difficult and arduous work. From 1861 onwards his papers show him dealing with the results of dredging in northern waters, so that in 1868, in the 'Shetland Final Dredging Report,' Part II, his share is "On the Crustacea, Tunicata, Polyzoa, Echinodermata, Actinozoa, Hydrozoa, and Porifera," invertebrate groups which few specialists would care to tackle collectively. In those days, it is true, the literature of all branches was not so crowded as it has since

A. M. Norman

become; but it was even then extensive, and not so very easy for a country clergyman to consult, much less to have at home in his own library. Later on, referring to this period, Norman spoke of the keen pleasure with which a necessary book was then acquired as compared with the unemotional acquisition which was a matter of course to a replenished purse.

Acquaintance with the present writer, which quickly ripened into intimacy and abiding friendship, was invited by a letter from Norman, as follows:—"Burnmoor Rectory, Fence Houses, Co. Durham, May 6, 1872. Dear Sir, We have so few carcinologists that it gives me great pleasure to welcome an addition to their number," with other obliging remarks. It will be easily understood how encouraging was such a notice from an acknowledged authority of long standing to one who was then a neophyte in systematic Zoology, in every need of experienced help and guidance, and little suspicious of the necessity for sifting good work from bad done by pioneers in all departments. After much intervening correspondence, in 1875 Norman was attracted away from his favourite northern seas to spend his summer holiday in Devon, receiving a welcome in Torquay on his way to Salcombe, the classic hunting-ground of Colonel Montagu. To attain this goal, instead of making the comfortable and picturesque journey by land, he carried his dredging apparatus on board a Brixham trawler, with the result that, on his applying for accommodation which had been recommended to him at Salcombe, the scared landlady would have no dealing with so disreputable-looking a visitor. Too late her penitent eyes recognised in the rejected lodger a scrupulously well-groomed parson. To such mishaps or misunderstandings enthusiastic nature-students cannot help being exposed. A more inconvenient trouble occurred at a later date. The sorting of miscellaneous specimens brought up by the dredge is often rather uneasy work on land; much more trying is it on board a small vessel out at sea. It is useful, therefore, to have a series of graded sieves, so that the minuter forms, which are sometimes the most important, may be readily separated from those of coarser build. To fit these one into the other for compactness in travel the uppermost with the largest mesh will have the smallest diameter. On one occasion Norman, just ready to start on his brief holiday, found that the constructor of this apparatus, instead of following instructions, had followed his "common sense," which taught him that the largest sieve should have the largest mesh, and that the smallest mesh naturally belonged to the smallest sieve. Unhappily, this theoretical improvement caused a very annoying delay while it was being reversed in the interest of practical convenience.

Though he published numerous treatises independently, Norman loved to associate himself with other naturalists in publication, fully as much to their advantage as to his own. Only on one occasion did this lead to any misunderstanding. The highly important 'Monograph of the Marine and Freshwater Ostracoda of the North Atlantic and of North-Western Europe,' by Brady and Norman, of which the first part was published in 1889, had been awaited with keen and pleasurable expectation by another colleague.

This was David Robertson, "the Naturalist of Cumbrae," who, with absolutely no initial advantages, had become a successful man of business, an ardent zoologist, and an exceptionally acute observer of marine life. When the work appeared, to which he had given unstinted (though not literary) service, his name was missing from the title-page. Notwithstanding his genial and modest temperament, the disappointment was perhaps never quite cured, though substantially solaced later on when the University of Glasgow made him an honorary Doctor of Laws. Generally, Norman was ready enough to give praise where it was due, as when he denominated the Norwegian Prof. G. O. Sars "the prince of carcinologists."

Among the distinctions which Norman himself held, it may be noticed that he received from the Institute of France the medal struck in honour of the exploring expeditions by the "Talisman" and the "Travailleur" in the Bay of Biscay. These he had joined by the invitation of the French Government, and his varied knowledge of oceanic species made his presence on board those vessels in the highest degree acceptable. When, in 1906, the gold medal of the Linnean Society was awarded him, the President, Prof. Herdman, gave an ample summary of the medallist's services to science, which is on record in the 'Proceedings' of that Society, and need scarcely be repeated here. It may, however, be noted that it begins with the year 1851, when at the age of twenty Norman published an account of the Mollusca of Oxfordshire. Years afterwards it was pleasant to find him President of the Conchological Society, and, while sharing his hospitality to the members, to join them in admiring and examining his noble conchological collection. Besides taking an active part in describing the results obtained by various exploring vessels, Norman was of essential service to Wyville Thomson and John Murray in their business of selecting the army of workers by whom the gigantic Report on the "Challenger" expedition was in twelve years completed. His dealing with the fourth volume of the 'Monograph of the British Spongiadæ' was a very unselfish affair, from a scientific point of view, which he thus explains, "In editing this posthumous volume of his valued friend, his aim has been simply to leave it as Dr. Bowerbank's work. To have attempted to indicate his own (Norman's) views would have been to remodel the whole, and the species would have had to be thrown into more numerous genera, defined on different principles, while, on the other hand, the number of so-called species would have been considerably reduced." Incidentally, the same preface observes that "a large number of the localities, to which the editor's initial is attached, will be found to be situated in the counties of Galway and Mayo, where a remarkably fine collection of sponges was obtained during a scientific expedition which Mr. D. Robertson, of Glasgow [and Cumbrae], and himself made to that part of Ireland in the summer of 1874."

Tributes to Norman's diversified knowledge are given by the competition for the use of his name in generic terminology. This was started by his friend Prof. G. S. Brady, who in 1866 named a genus of the Ostracoda *Normania*. This, however, a little while before had been named *Loxoconcha* by G. O. Sars. In 1868, Bowerbank gave the name *Normania* to a genus of sponges, which occupies a rather peculiar position, seeing that Norman, in whose paper Bowerbank's definition is incorporated, himself points out Brady's previous use of the same generic name. In 1870, Axel Boeck chose *Normania* to designate a genus ef Amphipoda, but this being evidently preoccupied, was changed by Jules Bonnier into *Normanion* in 1893. In 1880, Brady had established *Normanella* for one of the Copepoda, and, finally Dr. Harmer, in 'Nature' for November 7, 1918, after referring to the many services to science rendered by Norman, says: "Another of his specially noteworthy discoveries was the enigmatic encrusting organism obtained by him in the neighbourhood of Madeira, and afterwards named *Merlia normani* in his honour by Mr. R. Kirkpatrick."

In 1895 Norman was persuaded to migrate from Burnmoor to the far more important rectory of Houghton-le-Spring. The transfer of his innumerable and precious natural history specimens was a source of considerable anxiety. In his new sphere, besides being rural dean, he needed the help of three or even four curates, and though he delighted in horticulture, a rectory garden of seven acres was almost an embarrassment of riches. After a few years even his robust constitution felt the strain, so that during the present century he has resided at Berkhamsted, in a roomy house such as his library and collections required, but with a more manageable garden for retired leisure.

During this period, after waiting patiently but in vain for further material, from 1887 to 1912, he completed, so far as practicable, his account of the eccentric cirripede, *Synagoga mira*, which has been taken by Gruvel as type of a new family Synagogidæ. How little inclined he was by retiring to lead a life of indolence may be judged by various other publications. Among these is the important volume on the "Crustacea of Devon and Cornwall," in collaboration with Thomas Scott, LL.D., F.L.S. Furthermore, on February 15, 1907, he writes: "I am now engaged in preparing a paper on the Marine Mollusca of Madeira.... My joint report with Brady on the Crustacea of Northumberland and Durham is passing through the post. On April 27 I have to deliver a President's Address for the Herts Nat. Hist. Soc..... I am thinking of a paper with diagrams of types of freshwater Crustacea in the hope of stirring up some observers in the county..... I have just been instrumental in starting here a society of literature, science, and art.... Then there has just been started a clerical book club of which they unfortunately made me librarian." Among the results of his visit to Madeira in 1898, he had already published a short paper on the Land Isopods of that Island. The second presidential address which he delivered to the Hertfordshire Society at Watford, April 25, 1908, gave the inland students of science a refreshing story of maritime exploration, under the heading "The Celtic Province, its extent and its Marine Fauna." During the last few years the necessity for employing an amanuensis checked the flow of his correspondence. But apart from occasional sorrows, it seems true to say that his whole life was one of happiness rewarding virtue, the conscientious discharge of duty, the zeal for exploring and explaining the secrets of Nature, and the readiness for friendship and partnership in all his pursuits.

T. R. R. STEBBING.

INDEX TO VOL. XC. (B)

Abney (Sir W. de W.) Two Cases of Congenital Night-Blindness, 69.
Aggregation, investigations on state of (Schryver and Speer), 400.
Anaphylaxis, biological significance of (Dale), 556.
Anthocyanins and cyanidins, production of (Everest), 251.
Atmosphere, cooling and evaporative powers of (Hill and Hargood-Ash), 438.

Bacillus tetani, isolation and serological differentiation of (Tulloch), 145 : distribution of serological types of, in wounds, after inoculation (Tulloch), 529.
Bacterial infection, new factor in mechanism of (Bullock and Cramer), 513.
Bactericidal properties conferred on blood (Browning and Gulbransen), 136.
Balls (W. L.) The Existence of Daily Growth-Rings in the Cell Wall of Cotton Hairs, 542.
Bazett (H. C.) Observations on Changes in the Blood Pressure and Blood Volume following Operations in Man, 415.
Bell (R.) Obituary notice of, xxvi.
Beri-beri, distribution of deficiency substance of, in wheat, rice, and maize grains (Chick and Hume), 44.
Blood, bactericidal properties conferred on (Browning and Gulbransen), 136.
Blood pressure and volume, changes following operations (Bazett), 415.
Bose (Sir J. C.) and Das (G.) Researches on Growth and Movement in Plants by means of the High Magnification Crescograph, 364.
Bottomley (W. B.) The Isolation from Peat of certain Nucleic Acid Derivatives, 39.
Browning (C. H.) and Gulbransen (R.) Bactericidal Properties conferred on the Blood by Intravenous Injections of Diamino-Acridine Sulphate, 136.
Browning (C. H.) and Russ (S.) The Germicidal Action of Ultra-violet Radiation, and its Correlation with Selective Absorption, 33, 107.
Bullock (W. E.) and Cramer (W.) On a New Factor in the Mechanism of Bacterial Infection, 513.

Cannon (W. B.) Croonian Lecture—The Physiological Basis of Thirst, 283.
Chick (H.) and Hume (E. M.) The Distribution in Wheat, Rice, and Maize Grains of the Substance, the Deficiency of which in a Diet causes Polyneuritis in Birds and Beri-beri in Man, 44 ; —— The Effect of Exposure to Temperatures at or above 100° C. upon the Substance (Vitamine) whose Deficiency in a Diet causes Polyneuritis in Birds and Beri-beri in Man, 60.
Coal, four visible ingredients in (Stopes), 470.
Colloidal silica solutions, nature of growths in (Onslow), 266.
Cotton hairs, growth-rings in cell wall of (Balls), 542.
Cramer (W.). See Bullock and Cramer.
Crescograph, researches on growth in plants by means of the (Bose and Das), 364.
Croonian Lecture (Hickson), 108 ; (Cannon), 283 ; (Dale), 556.

Dale (H. H.) Croonian Lecture :—The Biological Significance of Anaphylaxis, 556.
Das (G.) See Bose and Das.
Dreyer (N. B.) and Sherrington (C. S.) Brevity, Frequency of Rhythm, and Amount of Reflex Nervous Discharge, as indicated by Reflex Contractions, 270.

Echinoderm larvæ with two and with no water-vascular systems, artificial production of (MacBride), 323.
Emotive physiological changes, measurement of (Waller), 214.
Enzymes in decomposition of glucose and mannitol by *B. coli communis* (Grey), 75, 92.
Everest (A. E.) The Production of Anthocyanins and Anthocyanidins.—Part III, 251.

Farmer (J. B.) On the Quantitative Differences in the Water-Conductivity of the Wood in Trees and Shrubs. Part I.—The Evergreens, 218; Part II.—The Deciduous Plants, 232.
Fibrillation in mammalian heart (MacWilliam), 302.
Flocculation of colloids by salts containing organic ions (Schryver and Speer), 400.

Geotropic stimulation and electrical conductivity (Small), 349.
Glucose and mannitol, decomposition by *B. coli communis* (Grey), 75, 92.
Greenwood (M.) On the Efficiency of Muscular Work, 199.
Grey (E. C.) The Enzymes concerned in the Decomposition of Glucose and Mannitol by *B. coli communis*. Part II.—Experiments of Short Duration with an Emulsion of the Organisms, 75; Part III.—Various Phases in the Decomposition of Glucose by an Emulsion of the Organisms, 92.
Growth and movement in Plants (Bose and Das), 364.
Gulbransen (R.) See Browning and Gulbransen.

Hargood-Ash (D.) See Hill and Hargood-Ash.
Heart, mechanism and control of fibrillation in (MacWilliam), 302.
Hickson (S. J.) Croonian Lecture: Evolution and Symmetry in the Order of the Sea-pens, 108.
Hill (L.) and Hargood-Ash (D.) On the Cooling and Evaporative Powers of the Atmosphere, as determined by the Kata-thermometer, 438.
Hull (E.) Obituary notice of, xxviii.
Hume (E. M.) See Chick and Hume.

Interference bands formed by rays at small angles (Mallock), 186.

Kata-thermometer, cooling and evaporation determined by (Hill and Hargood-Ash), 438.
Kidd (F.) See Stiles and Kidd.

Light rays, action on, and photosynthesis of, organic compounds (Moore and Webster), 168.
Lucas (K.) Obituary notice of, xxxi.

MacBride (E. W.) The Artificial Production of Echinoderm Larvæ with Two Water-Vascular Systems, and also of Larvæ Devoid of a Water-Vascular System, 323.
MacWilliam (J. A.) The Mechanism and Control of Fibrillation in the Mammalian Heart, 302.
Mallock (A.) Growth of Trees, with a Note on Interference Bands formed by Rays at Small Angles, 186.
Moore (B.) The Formation of Nitrites from Nitrates in Aqueous Solution by the Action of Sunlight, and the Assimilation of the Nitrites by Green Leaves in Sunlight, 158; —— and Webster (T. A.) Action of Light Rays on Organic Compounds, and the Photo-synthesis of Organic from Inorganic Compounds in Presence of Inorganic Colloids, 168.

Mottram (J. C.) and Russ (S.) Observations and Experiments on the Susceptibility and Immunity of Rats towards Jensen's Rat Sarcoma, 1.
Muscular work, efficiency of (Greenwood), 199.

Night-blindness, cases of congenital (Abney), 69.
Nitrites from nitrates in aqueous solution, formation of, by action of sunlight, and assimilation by green leaves (Moore), 158.
Norman (A. M.) Obituary notice of, xlvi.
Nucleic acid derivatives isolated from peat (Bottomley), 39.

Obituary Notices :—
- Power, Sir W. H., i.
- Reid, C., viii.
- Oliver, D., xi.
- Smith, Capt. G. W., xvi.
- Solms-Laubach, H. Graf zu, xix.
- Bell, R., xxvi.
- Hull, E., xxviii.
- Lucas, K., xxxi.
- Plimmer, H. G., xliii.
- Norman, A. M., xlvi.

Oliver (D.) Obituary notice of, xi.
Onslow (H.) Note on the Nature of Growths in Colloidal Silica Solutions, 266.

Peat, isolation of nucleic acid derivatives from (Bottomley), 39.
Photo-synthesis of organic compounds in presence of inorganic colloids (Moore and Webster), 168.
Plant cells, position of equilibrium in intake of salts by (Stiles and Kidd), 448.
Plants, growth and movement in (Bose and Das), 364.
Plant tissue, rate of absorption of salts by (Stiles and Kidd), 487.
Plimmer (H. G.) Obituary notice of, xliii.
Polyneuritis in birds, distribution in grains of deficiency substance of (Chick and Hume), 44 ; —— Effect of exposure to high temperatures upon the substance (Chick and Hume), 60.
Power (Sir W. H.) Obituary notice of, i.

Rat sarcoma, susceptibility and immunity of rats towards (Mottram and Russ), 1.
Reflex nervous discharge and reflex contraction (Dreyer and Sherrington), 270.
Reid (Clement) Obituary notice of, viii.
Russ (S.) See Mottram and Russ ; Browning and Russ.

Schryver (S. B.) and Speer (N. E.) Investigations dealing with the State of Aggregation. Part IV.—The Flocculation of Colloids by Salts containing Univalent Organic Ions, 400.
Scorbutic diet and teeth in guinea-pig (Zilva and Wells), 505.
Sea-pens, evolution and symmetry in the order of the (Hickson), 108.
Sherrington (C. S.) See Dreyer and Sherrington.
Small (J.) Changes of Electrical Conductivity under Geotropic Stimulation, 349.
Smith (Capt. G. W.) Obituary notice of, xvi.
Solms-Laubach (H. Graf zu) Obituary notice of, xix.
Speer (N. E.) See Schryver and Speer.
Stiles (W.) and Kidd (F.) The Influence of External Concentration on the Position of the Equilibrium attained in the Intake of Salts by Plant Cells, 448 ; —— The Comparative Rate of Absorption of Various Salts by Plant Tissue, 487.
Stopes (M. C.) On the Four Visible Ingredients in Banded Bituminous Coal : Studies in the Composition of Coal, No. 1, 470.

Teeth of guinea-pig, changes by scorbutic diet (Zilva and Wells), 505.
Tetanus, mechanism of infection in, and immunity from (Tulloch), 529.
Thirst, physiological basis of (Cannon), 283.
Trees and Shrubs, water-conductivity of wood in (Farmer), 218, 232.
Trees, growth of (Mallock), 186.
Tulloch (W. J.) The Isolation and Serological Differentiation of *Bacillus tetani*, 145 ; —— The Distribution of the Serological Types of *B. tetani* in Wounds of Men who received Prophylactic Inoculation, and a Study of the Mechanism of Infection in, and Immunity from, Tetanus, 529.

Ultra-violet radiation, germicidal action of (Browning and Russ), 33, 107.

Vitamines, distribution in grains, and effect of high temperatures on (Chick and Hume), 44, 60.

Waller (A. D.) The Galvanometric Measurement of "Emotive" Physiological Changes, 214.
Webster (T. A.) See Moore and Webster.
Wells (F. M.) See Zilva and Wells.
Wood in trees and shrubs, difference in water-conductivity of (Farmer), 218, 232.

Zilva (S. S.) and Wells (F. M.) Changes in the Teeth of the Guinea-Pig, produced by a Scorbutic Diet, 505.

END OF THE NINETIETH VOLUME (SERIES B).

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Page

ON THE ARC SPECTRUM OF SCANDIUM. By Sir William Crookes, O.M., D.Sc., F.R.S. (Plate 11) .. 438

VIBRATION AND STRENGTH OF STRUTS AND CONTINUOUS BEAMS UNDER END THRUSTS. By W. L. Cowley, A.R.C.Sc., D.I.C., and H. Levy, M.A., B.Sc., F.R.S.E. 440

TWO-DIMENSIONAL SOLUTIONS OF POISSON'S AND LAPLACE'S EQUATIONS.—Part I. By Leonard Bairstow, F.R.S., C.B.E., Part II. By Arthur Berry, M.A. 457

SCATTERING OF LIGHT BY SOLID SUBSTANCES. By the Hon. R. J. Strutt, F.R.S. (Plate 12) .. 476

A NEW METHOD OF WEIGHING COLLOIDAL PARTICLES. By E. F. Burton, Ph.D. .. 480

THE CONSTITUTION OF SULPHUR VAPOUR. By Sir James J. Dobbie, F.R.S., and J. J. Fox, M.B.E., D.Sc. (Plates 13 and 14) .. 484

OBITUARY NOTICES OF FELLOWS DECEASED:—Bertram Hopkinson (with portrait) .. xxvi—xxxvi

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